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(54) Title: PREVENTION AND TREATMENT OF AMYLOIDOGENIC DISEASE

(57) Abstract

The invention provides compositions and methods for treatment of amyloidogenic diseases. Such methods entail administering an agent that induces a beneficial immune response against an amyloid deposit in the patient. The methods are particularly useful for prophylactic and therapeutic treatment of Alzheimer's disease. In such methods, a suitable agent is Aβ peptide or an antibody thereto.

PREVENTION AND TREATMENT OF AMYLOIDOGENIC DISEASE

CROSS-REFERENCE TO RELATED APPLICATIONS

This application derives priority from USSN 60/067,740, filed December 2, 1997, and USSN 60/080,970, filed April 7, 1998, which are incorporated by reference in their entirety for all purposes.

5   TECHNICAL FIELD

The invention resides in the technical fields of immunology and medicine.

BACKGROUND

Alzheimer's disease (AD) is a progressive disease resulting
10   in senile dementia. *See generally* Selkoe, *TINS* 16, 403-409 (1993); Hardy et al., WO 92/13069; Selkoe, *J. Neuropathol. Exp. Neurol.* 53, 438-447 (1994); Duff et al., *Nature* 373, 476-477 (1995); Games et al., *Nature* 373, 523 (1995). Broadly speaking the disease falls into two categories: late onset,
15   which occurs in old age (65 + years) and early onset, which develops well before the senile period, i.e, between 35 and 60 years. In both types of disease, the pathology is the same but the abnormalities tend to be more severe and widespread in cases beginning at an earlier age. The disease is
20   characterized by two types of lesions in the brain, senile plaques and neurofibrillary tangles. Senile plaques are areas of disorganized neuropil up to 150 μm across with extracellular amyloid deposits at the center visible by microscopic analysis of sections of brain tissue.
25   Neurofibrillary tangles are intracellular deposits of tau protein consisting of two filaments twisted about each r in pairs.

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The principal constituent of the plaques is a peptide
termed Aβ or β-amyloid peptide. Aβ peptide is an internal
fragment of 39-43 amino acids of a precursor protein termed
amyloid precursor protein (APP). Several mutations within the
APP protein have been correlated with the presence of
Alzheimer's disease. See, e.g., Goate et al., *Nature* 349,
704) (1991) (valine[717] to isoleucine); Chartier Harlan et al.
Nature 353, 844 (1991)) (valine[717] to glycine); Murrell et
al., *Science* 254, 97 (1991) (valine[717] to phenylalanine);
Mullan et al., *Nature Genet.* 1, 345 (1992) (a double mutation
changing lysine[595]-methionine[596] to asparagine[595]-leucine[596]).
Such mutations are thought to cause Alzheimer's disease by
increased or altered processing of APP to Aβ, particularly
processing of APP to increased amounts of the long form of Aβ
(i.e., Aβ1-42 and Aβ1-43). Mutations in other genes, such as
the presenilin genes, PS1 and PS2, are thought indirectly to
affect processing of APP to generate increased amounts of long
form Aβ (see Hardy, *TINS* 20, 154 (1997)). These observations
indicate that Aβ, and particularly its long form, is a
causative element in Alzheimer's disease.

McMichael, EP 526,511, proposes administration of
homeopathic dosages (less than or equal to 10^{-2} mg/day) of Aβ
to patients with preestablished AD. In a typical human with
about 5 liters of plasma, even the upper limit of this dosage
would be expected to generate a concentration of no more than
2 pg/ml. The normal concentration of Aβ in human plasma is
typically in the range of 50-200 pg/ml (Seubert et al., *Nature*
359, 325-327 (1992)). Because EP 526,511's proposed dosage
would barely alter the level of endogenous circulating Aβ and
because EP 526,511 does not recommend use of an adjuvant, it
seems implausible that any therapeutic benefit would result.

By contrast, the present invention is directed *inter alia*
to treatment of Alzheimer's and other amyloidogenic diseases
by administration of Aβ or other immunogen to a patient under
conditions that generate a beneficial immune response in the
patient. The invention thus fulfills a longstanding need for
therapeutic regimes for preventing or ameliorating the
neuropathology of Alzheimer's disease.

SUMMARY OF THE CLAIMED INVENTION

In one aspect, the invention provides methods of preventing
or treating a disease characterized by amyloid deposition in a
patient. Such methods entail inducing an immune response
5 against a peptide component of an amyloid deposit in the
patient. Such induction can be active by administration of an
immunogen or passive by administration of an antibody or an
active fragment or derivative of the antibody. In some
patients, the amyloid deposit is aggregated Aβ peptide and
10 the disease is Alzheimer's disease. In some methods, the
patient is asymptomatic. In some methods, the patient is
under 50 years of age. In some methods, the patient has
inherited risk factors indicating susceptibility to
Alzheimer's disease. Such risk factors include variant
15 alleles in presenilin gene PS1 or PS2 and variant forms of
APP. In other methods, the patient has no known risk factors
for Alzheimer's disease.

For treatment of patients suffering from Alzheimer's
disease, one treatment regime entails administering a dose of
20 Aβ peptide to the patient to induce the immune response. In
some methods, the Aβ peptide is administered with an adjuvant
that enhances the immune response to the Aβ peptide. In some
methods, the adjuvant is alum. In some methods, the adjuvant
is MPL. The dose of Aβ peptide administered to the patient is
25 typically at least 1 or 10 μg, if administered with adjuvant,
and at least 50 μg if administered without adjuvant. In some
methods, the dose is at least 100 μg.

In some methods, the Aβ peptide is Aβ1-42. In some
methods, the Aβ peptide is administered in aggregated form.
30 In other methods, the Aβ peptide is administered in
dissociated form. In some methods, the therapeutic agent is
an effective dose of a nucleic acid encoding Aβ or an active
fragment or derivative thereof. The nucleic acid encoding Aβ
or fragment thereof is expressed in the patient to produce Aβ
35 or the active fragment thereof, which induces the immune
response. In some such methods, the nucleic acid is
administered through the skin, optionally via a patch. In
some methods, a therapeutic agent is identified by screening a

library of compounds to identify a compound reactive with
antibodies to Aβ, and administering the compound to the
patient to induce the immune response.

In some methods, the immune response is directed to
5   aggregated Aβ peptide without being directed to dissociated Aβ
peptide. For example, the immune response can comprise
antibodies that bind to aggregated Aβ peptide without binding
to dissociated Aβ peptide. In some methods, the immune
response comprises T-cells that bind to Aβ complexed with MCH1
10   or MHCII on CD8 or CD4 cells. In other methods, the immune
response is induced by administering an antibody to Aβ to the
patient. In some methods, the immune response is induced by
removing T-cells from the patient, contacting the T-cells with
Aβ peptide under conditions in which the T-cells are primed,
15   and replacing the T-cells in the patient.

The therapeutic agent is typically administered orally,
intranasally, intradermally, subcutaneously, intramuscularly,
topically or intravenously. In some methods, the patient is
monitored followed administration to assess the immune
20   response. If the monitoring indicates a reduction of the
immune response over time, the patient can be given one or
more further doses of the agent.

In another aspect, the invention provides pharmaceutical
compositions comprising Aβ and an excipient suitable for oral
25   and other routes of administration. The invention also
provides pharmaceutical compositions comprising an agent
effective to induce an immunogenic response against Aβ in a
patient, and a pharmaceutically acceptable adjuvant. In some
such compositions, the agent is Aβ or an active fragment
30   thereof. In some compositions, the adjuvant comprises alum.
In some compositions, the adjuvant comprises an oil-in-water
emulsion. In some compositions, the Aβ or active fragment is
a component of a polylactide polyglycolide copolymer (PLPG) or
other particle. The invention further provides compositions
35   comprising Aβ or an active fragment linked to a conjugate
molecule that promotes delivery of Aβ to the bloodstream of a
patient and/or promotes an immune response against Aβ. For
example, the conjugate can serve to promote an immune response

against Aβ.   In some compositions, the conjugate is cholera toxin.   In some compositions, the conjugate is an immunoglobulin.   In some compositions, the conjugate is attenuated diphtheria toxin CRM 197 (Gupta, *Vaccine* 15, 1341-3 (1997).

The invention also provides pharmaceutical compositions comprising an agent effect to induce an immunogenic response against Aβ in a patient with the proviso that the composition is free of Complete Freund's adjuvant.   The invention also provides compositions comprising a viral vector encoding Aβ or a an active fragment thereof effective to induce an immune response against Aβ.   Suitable viral vectors include herpes, adenovirus, adenoassociated virus, a retrovirus, sindbis, semiliki forest virus, vaccinia or avian pox.

The invention further provides methods of preventing or treating Alzheimer's disease.   In such methods, an effective dose of Aβ peptide is administered to a patient.   The invention further provides for the use of Aβ, or an antibody thereto, in the manufacture of a medicament for prevention or treatment of Alzheimer's disease.

In another aspect, the invention provides methods of assessing efficacy of an Alzheimer's treatment method in a patient.   In these methods, a baseline amount of antibody specific for Aβ peptide is determined in a tissue sample from the patient before treatment with an agent.   An amount of antibody specific for Aβ peptide in the tissue sample from the patient after treatment with the agent is compared to the baseline amount of Aβ peptide-specific antibody.   An amount of Aβ peptide-specific antibody measured after the treatment that is significantly greater than the baseline amount of Aβ peptide-specific antibody indicates a positive treatment outcome.

In others methods of assessing efficacy of an Alzheimer's treatment method in a patient, a baseline amount of antibody specific for Aβ peptide in a tissue sample from a patient before treatment with an agent is determined.   An amount of antibody specific for Aβ peptide in the tissue sample from the subject after treatment with the agent is compared to the

baseline amount of Aβ peptide-specific antibody. A reduction or lack of significant difference between the amount of Aβ peptide-specific antibody measured after the treatment compared to the baseline amount of Aβ peptide-specific antibody indicates a negative treatment outcome.

In other methods of assessing efficacy of an Alzheimer's disease treatment method in a patient a control amount of antibody specific for Aβ peptide is determined in tissue samples from a control population. An amount of antibody specific for Aβ peptide in a tissue sample from the patient after administering an agent is compared to the control amount of Aβ peptide-specific antibody. An amount of Aβ peptide-specific antibody measured after the treatment that is significantly greater than the control amount of Aβ peptide-specific antibody indicates a positive treatment outcome.

In other methods of assessing efficacy of an Alzheimer's treatment method in a patient, a control amount of antibody specific for Aβ peptide in tissues samples from a control population is determined. An amount of antibody specific for Aβ peptide in a tissue sample from the patient after administering an agent is compared to the control amount of Aβ peptide-specific antibody. A lack of significant difference between the amount of Aβ peptide-specific antibody measured after beginning said treatment compared to the control amount of.Aβ peptide-specific antibody indicates a negative treatment outcome.

Other methods of monitoring Alzheimer's disease or susceptibility thereto in a patient, comprise detecting an immune response against Aβ peptide in a sample from the patient. In some such methods, the patient is being administered an agent effective to treat or prevent Alzheimer's disease, and the level of the response determines the future treatment regime of the patient.

In other methods of assessing efficacy of an Alzheimer's treatment method in a patient a value for an amount of antibody specific for Aβ peptide in tissue sample from a patient who has been treated with an agent is determined. The

value is compared with a control value determined from a
population of patient experiencing amelioriation of, or
freedom from, symptoms of Alzheimer's disease due to treatment
with the agent. A value in the patient at least equal to the
5   control value indicates a positive response to treatment.

The invention further provides diagnostic kits for
performing the above methods. Such kits typically include a
reagent that specifically binds to antibodies to Aβ or which
stimulates proliferation of T-cells reactive with Aβ.

10   BRIEF DESCRIPTION OF THE FIGURES
Fig. 1: Antibody titer after injection of transgenic mice
with Aβ1-42.

Fig. 2: Amyloid burden in the hippocampus. The percentage
of the area of the hippocampal region occupied by amyloid
15   plaques, defined by reactivity with the Aβ-specific mAβ 3D6,
was determined by computer-assisted quantitative image
analysis of immunoreacted brain sections. The values for
individual mice are shown sorted by treatment group. The
horizontal line for each grouping indicates the median value
20   of the distribution.

Fig 3: Neuritic dystrophy in the hippocampus. The
percentage of the area of the hippocampal region occupied by
dystrophic neurites, defined by their reactivity with the
human APP-specific mAβ 8E5, was determined by quantitative
25   computer-assisted image analysis of immunoreacted brain
sections. The values for individual mice are shown for the
AN1792-treated group and the PBS-treated control group. The
horizontal line for each grouping indicates the median value
of the distribution.

30   Fig. 4: Astrocytosis in the retrosplenial cortex. The
percentage of the area of the cortical region occupied by
glial fibrillary acidic protein (GFAP)-positive astrocytes was
determined by quantitative computer-assisted image analysis of
immunoreacted brain sections. The values for individual mice
35   are shown sorted by treatment group and median group values
are indicated by horizontal lines.

Fig. 5: Geometric mean antibody titers to Aβ1-42 following immunization with a range of eight doses of AN1792 containing 0.14, 0.4, 1.2, 3.7, 11, 33, 100, or 300 µg.

Fig. 6: Kinetics of antibody response to AN1792
5   immunization. Titers are expressed as geometric means of values for the 6 animals in each group.

Fig. 7: Quantitative image analysis of the cortical amyloid burden in PBS- and AN1792-treated mice.

Fig. 8: Quantitative image analysis of the neuritic plaque
10   burden in PBS- and AN1792-treated mice.

Fig. 9: Quantitative image analysis of the percent of the retrosplenial cortex occupied by astrocytosis in PBS- and AN1792-treated mice.

Fig. 10: Lymphocyte Proliferation Assay on spleen cells
15   from AN1792-treated (upper panel) or PBS-treated (lower panel).

Fig. 11: Total Aβ levels in the cortex. A scatterplot of individual Aβ profiles in mice immunized with Aβ or APP derivatives combined with Freund's adjuvant.

20   Fig. 12: Amyloid burden in the cortex was determined by quantitative image analysis of immunoreacted brain sections for mice immunized with the Aβ peptide conjugates Aβ1-5, Aβ1-12, and Aβ13-28; the full length Aβ aggregates AN1792 (Aβ1-42) and AN1528 (Aβ1-40) and the PBS-treated control group.

25   Fig. 13: Geometric mean titers of Aβ-specific antibody for groups of mice immunized with Aβ or APP derivatives combined with Freund's adjuvant.

Fig. 14: Geometric mean titers of Aβ-specific antibody for groups of guinea pigs immunized with AN1792, or a
30   palmitoylated derivative thereof, combined with various adjuvants.

Fig: 15: Aβ levels in the cortex of 12-month old PDAPP mice treated with AN1792 or AN1528 with different adjuvants.

DEFINITIONS

35   The term "substantial identity" means that two peptide sequences, when optimally aligned, such as by the programs GAP or BESTFIT using default gap weights, share at least 65

percent sequence identity, preferably at least 80 or 90
percent sequence identity, more preferably at least 95 percent
sequence identity or more (e.g., 99 percent sequence identity
or higher). Preferably, residue positions which are not
5   identical differ by conservative amino acid substitutions.
     For sequence comparison, typically one sequence acts as a
reference sequence, to which test sequences are compared.
When using a sequence comparison algorithm, test and reference
sequences are input into a computer, subsequence coordinates
10   are designated, if necessary, and sequence algorithm program
parameters are designated. The sequence comparison algorithm
then calculates the percent sequence identity for the test
sequence(s) relative to the reference sequence, based on the
designated program parameters.
15   Optimal alignment of sequences for comparison can be
conducted, e.g., by the local homology algorithm of Smith &
Waterman, *Adv. Appl. Math.* 2:482 (1981), by the homology
alignment algorithm of Needleman & Wunsch, *J. Mol. Biol.*
48:443 (1970), by the search for similarity method of Pearson
20   & Lipman, *Proc. Nat'l. Acad. Sci. USA* 85:2444 (1988), by
computerized implementations of these algorithms (GAP,
BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software
Package, Genetics Computer Group, 575 Science Dr., Madison,
WI), or by visual inspection (*see generally* Ausubel *et al.*,
25   *supra*). One example of algorithm that is suitable for
determining percent sequence identity and sequence similarity
is the BLAST algorithm, which is described in Altschul *et al.*,
J. Mol. Biol. 215:403-410 (1990). Software for performing
BLAST analyses is publicly available through the National
30   Center for Biotechnology Information
(http://www.ncbi.nlm.nih.gov/). Typically, default program
parameters can be used to perform the sequence comparison,
although customized parameters can also be used. For amino
acid sequences, the BLASTP program uses as defaults a
35   wordlength (W) of 3, an expectation (E) of 10, and the
BLOSUM62 scoring matrix (*see* Henikoff & Henikoff, *Proc. Natl.
Acad. Sci. USA* 89, 10915 (1989))

For purposes of classifying amino acids substitutions as conservative or nonconservative, amino acids are grouped as follows: Group I (hydrophobic sidechains): norleucine, met, ala, val, leu, ile; Group II (neutral hydrophilic side chains): cys, ser, thr; Group III (acidic side chains): asp, glu; Group IV (basic side chains): asn, gln, his, lys, arg; Group V (residues influencing chain orientation): gly, pro; and Group VI (aromatic side chains): trp, tyr, phe. Conservative substitutions involve substitutions between amino acids in the same class. Non-conservative substitutions constitute exchanging a member of one of these classes for a member of another.

Therapeutic agents of the invention are typically substantially pure. This means that an agent is typically at least about 50% w/w (weight/weight) purity, as well as being substantially free from interfering proteins and contaminants. Sometimes the agents are at least about 80% w/w and, more preferably at least 90 or about 95% w/w purity. However, using conventional protein purification techniques, homogeneous peptides of at least 99% w/w can be obtained.

Specific binding between two entities means an affinity of at least 10^6, 10^7, 10^8, 10^9 M^{-1}, or 10^{10} M^{-1}. Affinities greater than 10^8 M^{-1} are preferred.

The term "antibody" is used to include intact antibodies and binding fragments thereof. Typically, fragments compete with the intact antibody from which they were derived for specific binding to an antigen. Optionally, antibodies or binding fragments thereof, can be chemically conjugated to, or expressed as, fusion proteins with other proteins.

APP[695], APP[751], and APP[770] refer, respectively, to the 695, 751, and 770 amino acid residue long polypeptides encoded by the human APP gene. See Kang et al., *Nature* 325, 773 (1987); Ponte et al., *Nature* 331, 525 (1988); and Kitaguchi et al., *Nature* 331, 530 (1988). Amino acids within the human amyloid precursor protein (APP) are assigned numbers according to the sequence of the APP770 isoform. Terms such as Aβ39, Aβ40, Aβ41, Aβ42 and Aβ43 refer to an Aβ peptide containing amino acid residues 1-39, 1-40, 1-41, 1-42 and 1-43.

The term "epitope" or "antigenic determinant" refers to a site on an antigen to which B and/or T cells respond. B-cell epitopes can be formed both from contiguous amino acids or noncontiguous amino acids juxtaposed by tertiary folding of a

5   protein. Epitopes formed from contiguous amino acids are typically retained on exposure to denaturing solvents whereas epitopes formed by tertiary folding are typically lost on treatment with denaturing solvents. An epitope typically includes at least 3, and more usually, at least 5 or 8-10

10   amino acids in a unique spatial conformation. Methods of determining spatial conformation of epitopes include, for example, x-ray crystallography and 2-dimensional nuclear magnetic resonance. See, e.g., *Epitope Mapping Protocols in Methods in Molecular Biology*, Vol. 66, Glenn E. Morris, Ed.

15   (1996). Antibodies that recognize the same epitope can be identified in a simple immunoassay showing the ability of one antibody to block the binding of another antibody to a target antigen. T-cells recognize continuous epitopes of about nine amino acids for CD8 cells or about 13-15 amino acids for CD4

20   cells. T cells that recognize the epitope can be identified by *in vitro* assays that measure antigen-dependent proliferation, as determined by ^{3}H-thymidine incorporation by primed T cells in response to an epitope (Burke et al., *J. Inf. Dis.* 170, 1110-19 (1994)), by antigen-dependent killing

25   (cytotoxic T lymphocyte assay, Tigges et al., *J. Immunol.* 156, 3901-3910) or by cytokine secretion.

The term "immunological" or "immune" response is the development of a beneficial humoral (antibody mediated) and/or a cellular (mediated by antigen-specific T cells or their

30   secretion products) response directed against an amyloid peptide in a recipient patient. Such a response can be an active response induced by administration of immunogen or a passive response induced by administration of antibody or primed T-cells. A cellular immune response is elicited by the

35   presentation of polypeptide epitopes in association with Class I or Class II MHC molecules to activate antigen-specific CD4$^+$ T helper cells and/or CD8$^+$ cytotoxic T cells. The response may also involve activation of monocytes, macrophages, NK

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cells, basophils, dendritic cells, astrocytes, microglia cells, eosinophils or other components of innate immunity. The presence of a cell-mediated immunological response can be determined by proliferation assays ($CD4^+$ T cells) or CTL (cytotoxic T lymphocyte) assays (see Burke, supra; Tigges, supra). The relative contributions of humoral and cellular responses to the protective or therapeutic effect of an immunogen can be distinguished by separately isolating IgG and T-cells from an immunized syngeneic animal and measuring protective or therapeutic effect in a second subject.

An "immunogenic agent" or "immunogen" is capable of inducing an immunological response against itself on administration to a patient, optionally in conjunction with an adjuvant.

The term "naked polynucleotide" refers to a polynucleotide not complexed with colloidal materials. Naked polynucleotides are sometimes cloned in a plasmid vector.

The term "adjuvant" refers to a compound that when administered in conjunction with an antigen augments the immune response to the antigen, but when administered alone does not generate an immune response to the antigen. Adjuvants can augment an immune response by several mechanisms including lymphocyte recruitment, stimulation of B and/or T cells, and stimulation of macrophages.

The term "patient" includes human and other mammalian subjects that receive either prophylactic or therapeutic treatment.

Disaggregated or monomeric Aβ means soluble, monomeric peptide units of Aβ. One method to prepare monomeric Aβ is to dissolve lyophilized peptide in neat DMSO with sonication. The resulting solution is centrifuged to remove any nonsoluble particulates. Aggregated Aβ is a mixture of oligomers in which the monomeric units are held together by noncovalent bonds.

Compositions or methods "comprising" one or more recited elements may include other elements not specifically recited. For example, a composition that comprises Aβ peptide

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encompasses both an isolated Aβ peptide and Aβ peptide as a
component of a larger polypeptide sequence.

DETAILED DESCRIPTION

I. General

5   The invention provides pharmaceutical compositions and
methods for prophylactic and therapeutic treatment of diseases
characterized by accumulation of amyloid deposits. Amyloid
deposits comprise a peptide aggregated to an insoluble mass.
The nature of the peptide varies in different diseases but in
10   most cases, the aggregate has a β-pleated sheet structure and
stains with Congo Red dye. Diseases characterized by amyloid
deposits include Alzheimer's disease (AD), both late and early
onset. In both diseases, the amyloid deposit comprises a
peptide termed Aβ, which accumulates in the brain of affected
15   individuals. Examples of some other diseases characterized by
amyloid deposits are SAA amyloidosis, hereditary Icelandic
syndrome, multiple myeloma, and spongiform encephalopathies,
including mad cow disease, Creutzfeldt Jakob disease, sheep
scrapie, and mink spongiform encephalopathy (see Weissmann et
20   al., *Curr. Opin. Neurobiol.* 7, 695-700 (1997); Smits et al.,
Veterinary Quarterly 19, 101-105 (1997); Nathanson et al., Am.
J. Epidemiol. 145, 959-969 (1997)). The peptides forming the
aggregates in these diseases are serum amyloid A, cystantin C,
IgG kappa light chain respectively for the first three, and
25   prion protein for the others.

II. Therapeutic Agents

1. Alzheimer's Disease

Therapeutic agents for use in the present invention induce
an immune response against Aβ peptide. These agents include
30   Aβ peptide itself and variants thereof, analogs and mimetics
of Aβ peptide that induce and/or crossreact with antibodies to
Aβ peptide, and antibodies or T-cells reactive with Aβ
peptide. Induction of an immune response can be active as
when an immunogen is administered to induce antibodies or T-
35   cells reactive with Aβ in a patient, or passive, as when an
antibody is administered that itself binds to Aβ in patient.

Aβ, also known as β-amyloid peptide, or A4 peptide (see US 4,666,829; Glenner & Wong, *Biochem. Biophys. Res. Commun.* 120, 1131 (1984)), is a peptide of 39-43 amino acids, which is the principal component of characteristic plaques of Alzheimer's
5   disease. Aβ is generated by processing of a larger protein APP by two enzymes, termed β and γ secretases (see Hardy, *TINS* 20, 154 (1997)). Known mutations in APP associated with Alzheimer's disease occur proximate to the site of β or γ secretase, or within Aβ. For example, position 717 is
10   proximate to the site of γ-secretase cleavage of APP in its processing to Aβ, and positions 670/671 are proximate to the site of β-secretase cleavage. It is believed that the mutations cause AD disease by interacting with the cleavage reactions by which Aβ is formed so as to increase the amount
15   of the 42/43 amino acid form of Aβ generated.

A β has the unusual property that it can fix and activate both classical and alternate complement cascades. In particular, it binds to C1q and ultimately to C3bi. This association facilitates binding to macrophages leading to
20   activation of B cells. In addition, C3bi breaks down further and then binds to CR2 on B cells in a T cell dependent manner leading to a 10,000 increase in activation of these cells. This mechanism causes Aβ to generate an immune response in excess of that of other antigens.

25   The therapeutic agent used in the claimed methods can be any of the naturally occurring forms of Aβ peptide, and particularly the human forms (i.e., Aβ39, Aβ40, Aβ41, Aβ42 or Aβ43). The sequences of these peptides and their relationship to the APP precursor are illustrated by Fig. 1 of Hardy et
30   al., *TINS* 20, 155-158 (1997). For example, Aβ42 has the sequence:

    H_2N-Asp-Ala-Glu-Phe-Arg-His-Asp-Ser-Gly-Tyr-Glu-Val-His-His-
    Gln-Lys-Leu-Val-Phe-Phe-Ala-Glu-Asp-Val-Gly-Ser-Asn-Lys-Gly-
    Ala-Ile-Ile-Gly-Leu-Met-Val-Gly-Gly-Val-Val-IIe-Ala-OH.

35   Aβ41, Aβ40 and Aβ39 differ from Aβ42 by the omission of Ala, Ala-Ile, and Ala-Ile-Val respectively from the C-terminal end. Aβ43 differs from Aβ42 by the presence of a threonine residue

at the C-terminus. The therapeutic agent can also be an
active fragment or analog of a natural Aβ peptide that
contains an epitope that induces a similar protective or
therapeutic immune response on administration to a human.

5   Immunogenic fragments typically have a sequence of at least 3,
5, 6, 10 or 20 contiguous amino acids from a natural peptide.
Immunogenic fragments include Aβ1-5, 1-6, 1-12, 13-28, 17-28,
25-25, 35-40 and 35-42. Fragments from the N-terminal half of
Aβ are preferred in some methods. Analogs include allelic,

10 species and induced variants. Analogs typically differ from
naturally occurring peptides at one or a few positions, often
by virtue of conservative substitutions. Analogs typically
exhibit at least 80 or 90% sequence identity with natural
peptides. Some analogs also include unnatural amino acids or

15 modifications of N or C terminal amino acids. Examples of
unnatural amino acids are α,α-disubstituted amino acids, N-
alkyl amino acids, lactic acid, 4-hydroxyproline, γ-
carboxyglutamate, ε-N,N,N-trimethyllysine, ε-N-acetyllysine,
O-phosphoserine, N-acetylserine, N-formylmethionine, 3-

20 methylhistidine, 5-hydroxylysine, ω-N-methylarginine.
Fragments and analogs can be screened for prophylactic or
therapeutic efficacy in transgenic animal models as described
below.

Aβ, its fragments, analogs and other amyloidogenic peptides

25 can be synthesized by solid phase peptide synthesis or
recombinant expression, or can be obtained from natural
sources. Automatic peptide synthesizers are commercially
available from numerous suppliers, such as Applied Biosystems,
Foster City, California. Recombinant expression can be in

30 bacteria, such as E. coli, yeast, insect cells or mammalian
cells. Procedures for recombinant expression are described by
Sambrook et al., *Molecular Cloning: A Laboratory Manual*
(C.S.H.P. Press, NY 2d ed., 1989). Some forms of Aβ peptide
are also available commercially (e.g., American Peptides

35 Company, Inc., Sunnyvale, CA and California Peptide Research,
Inc. Napa, CA).

Therapeutic agents also include longer polypeptides that
include, for example, an Aβ peptide, active fragment or analog

together with other amino acids. For example, Aβ peptide can be present as intact APP protein or a segment thereof, such as the C-100 fragment that begins at the N-terminus of Aβ and continues to the end of APP. Such polypeptides can be

5   screened for prophylactic or therapeutic efficacy in animal models as described below. The Aβ peptide, analog, active fragment or other polypeptide can be administered in associated form (i.e., as an amyloid peptide) or in dissociated form. Therapeutic agents also include multimers

10   of monomeric immunogenic agents.

       In a further variation, an immunogenic peptide, such as Aβ, can be presented as a viral or bacterial vaccine. A nucleic acid encoding the immunogenic peptide is incorporated into a genome or episome of the virus or bacteria. Optionally, the

15   nucleic acid is incorporated in such a manner that the immunogenic peptide is expressed as a secreted protein or as a fusion protein with an outersurface protein of a virus or a transmembrane protein of a bacteria so that the peptide is displayed. Viruses or bacteria used in such methods should

20   be nonpathogenic or attenuated. Suitable viruses include adenovirus, HSV, vaccinia and fowl pox. Fusion of an immunogenic peptide to HBsAg of HBV is particularly suitable. Therapeutic agents also include peptides and other compounds that do not necessarily have a significant amino acid sequence

25   similarity with Aβ but nevertheless serve as mimetics of Aβ and induce a similar immune response. For example, any peptides and proteins forming β-pleated sheets can be screened for suitability. Anti-idiotypic antibodies against monoclonal antibodies to Aβ or other amyloidogenic peptides can also be

30   used. Such anti-Id antibodies mimic the antigen and generate an immune response to it (see Essential Immunology (Roit ed., Blackwell Scientific Publications, Palo Alto, 6th ed.), p. 181).

       Random libraries of peptides or other compounds can also be

35   screened for suitability. Combinatorial libraries can be produced for many types of compounds that can be synthesized in a step-by-step fashion. Such compounds include polypeptides, beta-turn mimetics, polysaccharides,

phospholipids, hormones, prostaglandins, steroids, aromatic
compounds, heterocyclic compounds, benzodiazepines, oligomeric
N-substituted glycines and oligocarbamates. Large
combinatorial libraries of the compounds can be constructed by
5   the encoded synthetic libraries (ESL) method described in
Affymax, WO 95/12608, Affymax, WO 93/06121, Columbia
University, WO 94/08051, Pharmacopeia, WO 95/35503 and
Scripps, WO 95/30642 (each of which is incorporated by
reference for all purposes). Peptide libraries can also be
10   generated by phage display methods. *See, e.g.,* Devlin, WO
91/18980.

Combinatorial libraries and other compounds are initially
screened for suitability by determining their capacity to bind
to antibodies or lymphocytes (B or T) known to be specific for
15   Aβ or other amyloidogenic peptides. For example, initial
screens can be performed with any polyclonal sera or
monoclonal antibody to Aβ or other amyloidogenic peptide.
Compounds identified by such screens are then further analyzed
for capacity to induce antibodies or reactive lymphocytes to
20   Aβ or other amyloidogenic peptide. For example, multiple
dilutions of sera can be tested on microtiter plates that have
been precoated with Aβ peptide and a standard ELISA can be
performed to test for reactive antibodies to Aβ. Compounds
can then be tested for prophylactic and therapeutic efficacy
25   in transgenic animals predisposed to an amyloidogenic disease,
as described in the Examples. Such animals include, for
example, mice bearing a 717 mutation of APP described by Games
et al., *supra,* and mice bearing a Swedish mutation of APP such
as described by McConlogue et al., US 5,612,486 and Hsiao et
30   al., *Science* 274, 99 (1996); Staufenbiel et al., *Proc. Natl.
Acad. Sci. USA* 94, 13287-13292 (1997); Sturchler-Pierrat et
al., *Proc. Natl. Acad. Sci. USA* 94, 13287-13292 (1997);
Borchelt et al., *Neuron* 19, 939-945 (1997)). The same
screening approach can be used on other potential agents such
35   as fragments of Aβ, analogs of Aβ and longer peptides
including Aβ, described above.

Therapeutic agents of the invention also include antibodies
that specifically bind to Aβ. Such antibodies can be

monoclonal or polyclonal. Some such antibodies bind specifically to the aggregated form of Aβ without binding to the dissociated form. Some bind specifically to the dissociated form without binding to the aggregated form. Some bind to both aggregated and dissociated forms. The production of non-human monoclonal antibodies, *e.g.*, murine or rat, can be accomplished by, for example, immunizing the animal with Aβ. *See* Harlow & Lane, *Antibodies, A Laboratory Manual* (CSHP NY, 1988) (incorporated by reference for all purposes). Such an immunogen can be obtained from a natural source, by peptides synthesis or by recombinant expression.

Humanized forms of mouse antibodies can be generated by linking the CDR regions of non-human antibodies to human constant regions by recombinant DNA techniques. *See* Queen et al., *Proc. Natl. Acad. Sci. USA* 86, 10029-10033 (1989) and WO 90/07861 (incorporated by reference for all purposes).

Human antibodies can be obtained using phage-display methods. *See, e.g.*, Dower et al., WO 91/17271; McCafferty et al., WO 92/01047. In these methods, libraries of phage are produced in which members display different antibodies on their outersurfaces. Antibodies are usually displayed as Fv or Fab fragments. Phage displaying antibodies with a desired specificity are selected by affinity enrichment to Aβ, or fragments thereof. Human antibodies against Aβ can also be produced from non-human transgenic mammals having transgenes encoding at least a segment of the human immunoglobulin locus and an inactivated endogenous immunoglobulin locus. *See, e.g.*, Lonberg et al., WO93/12227 (1993); Kucherlapati, WO 91/10741 (1991) (each of which is incorporated by reference in its entirety for all purposes). Human antibodies can be selected by competitive binding experiments, or otherwise, to have the same epitope specificity as a particular mouse antibody. Such antibodies are particularly likely to share the useful functional properties of the mouse antibodies. Human polyclonal antibodies can also be provided in the form of serum from humans immunized with an immunogenic agent. Optionally, such polyclonal antibodies can be concentrated by

affinity purification using Aβ or other amyloid peptide as an affinity reagent.

Human or humanized antibodies can be designed to have IgG, IgD, IgA and IgE constant region, and any isotype, including IgG1, IgG2, IgG3 and IgG4. Antibodies can be expressed as tetramers containing two light and two heavy chains, as separate heavy chains, light chains, as Fab, Fab' $F(ab')_2$, and Fv, or as single chain antibodies in which heavy and light chain variable domains are linked through a spacer.

Therapeutic agents for use in the present methods also include T-cells that bind to Aβ peptide. For example, T-cells can be activated against Aβ peptide by expressing a human MHC class I gene and a human β-2-microglobulin gene from an insect cell line, whereby an empty complex is formed on the surface of the cells and can bind to Aβ peptide. T-cells contacted with the cell line become specifically activated against the peptide. See Peterson et al., US 5,314,813. Insect cell lines expressing an MHC class II antigen can similarly be used to activate CD4 T cells.

2. Other Diseases

The same or analogous principles determine production of therapeutic agents for treatment of other amyloidogenic diseases. In general, the agents noted above for use in treatment of Alzheimer's disease can also be used for treatment early onset Alzheimer's disease associated with Down's syndrome. In mad cow disease, prion peptide, active fragments, and analogs, and antibodies to prion peptide are used in place of Aβ peptide, active fragments, analogs and antibodies to Aβ peptide in treatment of Alzheimer's disease. In treatment of multiple myeloma, IgG light chain and analogs and antibodies thereto are used, and so forth in other diseases.

3. Carrier Proteins

Some agents for inducing an immune response contain the appropriate epitope for inducing an immune response against amyloid deposits but are too small to be immunogenic. In this

situation, a peptide immunogen can be linked to a suitable carrier to help elicit an immune response. Suitable carriers include serum albumins, keyhole limpet hemocyanin, immunoglobulin molecules, thyroglobulin, ovalbumin, tetanus

5   toxoid, or a toxoid from other pathogenic bacteria, such as diphtheria, *E. coli*, cholera, or *H. pylori*, or an attenuated toxin derivative. Other carriers for stimulating or enhancing an immune response include cytokines such as IL-1, IL-1 α and β peptides, IL-2, γINF, IL-10, GM-CSF, and chemokines, such as

10   M1P1α and β and RANTES. Immunogenic agents can also be linked to peptides that enhance transport across tissues, as described in O'Mahony, WO 97/17613 and WO 97/17614.

Immunogenic agents can be linked to carriers by chemical crosslinking. Techniques for linking an immunogen to a

15   carrier include the formation of disulfide linkages using N-succinimidyl-3-(2-pyridyl-thio) propionate (SPDP) and succinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate (SMCC) (if the peptide lacks a sulfhydryl group, this can be provided by addition of a cysteine residue). These reagents

20   create a disulfide linkage between themselves and peptide cysteine resides on one protein and an amide linkage through the ϵ-amino on a lysine, or other free amino group in other amino acids. A variety of such disulfide/amide-forming agents are described by *Immun. Rev.* 62, 185 (1982). Other

25   bifunctional coupling agents form a thioether rather than a disulfide linkage. Many of these thio-ether-forming agents are commercially available and include reactive esters of 6-maleimidocaproic acid, 2-bromoacetic acid, and 2-iodoacetic acid, 4-(N-maleimido-methyl)cyclohexane-1-carboxylic acid.

30   The carboxyl groups can be activated by combining them with succinimide or 1-hydroxyl-2-nitro-4-sulfonic acid, sodium salt.

Immunogenic peptides can also be expressed as fusion proteins with carriers. The immunogenic peptide can be linked

35   at the amino terminus, the carboxyl terminus, or internally to the carrier. Optionally, multiple repeats of the immunogenic peptide can be present in the fusion protein.

4. Nucleic Acid Encoding Immunogens

Immune responses against amyloid deposits can also be induced by administration of nucleic acids encoding Aβ peptide or other peptide immunogens. Such nucleic acids can be DNA or RNA. A nucleic acid segment encoding the immunogen is typically linked to regulatory elements, such as a promoter and enhancer, that allow expression of the DNA segment in the intended target cells of a patient. For expression in blood cells, as is desirable for induction of an immune response, promoter and enhancer elements from light or heavy chain immunoglobulin genes or the CMV major intermediate early promoter and enhancer are suitable to direct expression. The linked regulatory elements and coding sequences are often cloned into a vector.

A number of viral vector systems are available including retroviral systems (see, e.g., Lawrie and Tumin, *Cur. Opin. Genet. Develop.* 3, 102-109 (1993)); adenoviral vectors (see, e.g., Bett et al., *J. Virol.* 67, 5911 (1993)); adeno-associated virus vectors (see, e.g., Zhou et al., *J. Exp. Med.* 179, 1867 (1994)), viral vectors from the pox family including vaccinia virus and the avian pox viruses, viral vectors from the alpha virus genus such as those derived from Sindbis and Semliki Forest Viruses (see, e.g., Dubensky et al., *J. Virol.* 70, 508-519 (1996)), and papillomaviruses (Ohe et al., *Human Gene Therapy* 6, 325-333 (1995); Woo et al., WO 94/12629 and Xiao & Brandsma, *Nucleic Acids. Res.* 24, 2630-2622 (1996)).

DNA encoding an immunogen, or a vector containing the same, can be packaged into liposomes. Suitable lipids and related analogs are described by US 5,208,036, 5,264,618, 5,279,833 and 5,283,185. Vectors and DNA encoding an immunogen can also be adsorbed to or associated with particulate carriers, examples of which include polymethyl methacrylate polymers and polylactides and poly(lactide-co-glycolides), see, e.g., McGee et al., *J. Micro Encap.* (1996).

Gene therapy vectors or naked DNA can be delivered *in vivo* by administration to an individual patient, typically by systemic administration (e.g., intravenous, intraperitoneal, nasal, gastric, intradermal, intramuscular, subdermal, or

intracranial infusion) or topical application (see e.g.,
US 5,399,346). DNA can also be administered using a gene
gun. See Xiao & Brandsma, *supra*. The DNA encoding an
immunogen is precipitated onto the surface of microscopic
5   metal beads. The microprojectiles are accelerated with a
shock wave or expanding helium gas, and penetrate tissues to a
depth of several cell layers. For example, The Accel™ Gene
Delivery Device manufactured by Agacetus, Inc. Middleton WI is
suitable. Alternatively, naked DNA can pass through skin into
10   the blood stream simply by spotting the DNA onto skin with
chemical or mechanical irritation (see WO 95/05853).
         In a further variation, vectors encoding immunogens can be
delivered to cells *ex vivo*, such as cells explanted from an
individual patient (e.g., lymphocytes, bone marrow aspirates,
15   tissue biopsy) or universal donor hematopoietic stem cells,
followed by reimplantation of the cells into a patient,
usually after selection for cells which have incorporated the
vector.

         III.   Patients Amenable to Treatment
20   Patients amenable to treatment include individuals at risk
of disease but not showing symptoms, as well as patients
presently showing symptoms. In the case of Alzheimer's
disease, virtually anyone is at risk of suffering from
Alzheimer's disease if he or she lives long enough.
25   Therefore, the present methods can be administered
prophylactically to the general population without any
assessment of the risk of the subject patient. The present
methods are especially useful for individuals who do have a
known genetic risk of Alzheimer's disease. Such individuals
30   include those having relatives who have experienced this
disease, and those whose risk is determined by analysis of
genetic or biochemical markers. Genetic markers of risk
toward Alzheimer's disease include mutations in the APP gene,
particularly mutations at position 717 and positions 670 and
35   671 referred to as the Hardy and Swedish mutations
respectively (see Hardy, *TINS*, *supra*). Other markers of risk
are mutations in the presenilin genes, PS1 and PS2, and ApoE4,

family history of AD, hypercholesterolemia or atherosclerosis.
Individuals presently suffering from Alzheimer's disease can
be recognized from characteristic dementia, as well as the
presence of risk factors described above. In addition, a

5   number of diagnostic tests are available for identifying
individuals who have AD. These include measurement of CSF tau
and Aβ42 levels. Elevated tau and decreased Aβ42 levels
signify the presence of AD. Individuals suffering from
Alzheimer's disease can also be diagnosed by MMSE or ADRDA

10 criteria as discussed in the Examples section.

     In asymptomatic patients, treatment can begin at any age
(e.g., 10, 20, 30). Usually, however, it is not necessary to
begin treatment until a patient reaches 40, 50, 60 or 70.
Treatment typically entails multiple dosages over a period of

15 time. Treatment can be monitored by assaying antibody, or
activated T-cell or B-cell responses to the therapeutic agent
(e.g., Aβ peptide) over time. If the response falls, a
booster dosage is indicated. In the case of potential Down's
syndrome patients, treatment can begin antenatally by

20 administering therapeutic agent to the mother or shortly after
birth.

IV.   Treatment Regimes

     In prophylactic applications, pharmaceutical compositions
or medicants are administered to a patient susceptible to, or

25 otherwise at risk of, a particular disease in an amount
sufficient to eliminate or reduce the risk or delay the outset
of the disease. In therapeutic applications, compositions or
medicants are administered to a patient suspected of, or.
already suffering from such a disease in an amount sufficient

30 to cure, or at least partially arrest, the symptoms of the
disease and its complications. An amount adequate to
accomplish this is defined as a therapeutically- or
pharmaceutically-effective dose. In both prophylactic and
therapeutic regimes, agents are usually administered in

35 several dosages until a sufficient immune response has been
achieved. Typically, the immune response is monitored and

repeated dosages are given if the immune response starts to fade.

Effective doses of the compositions of the present invention, for the treatment of the above described conditions vary depending upon many different factors, including means of administration, target site, physiological state of the patient, whether the patient is human or an animal, other medications administered, and whether treatment is prophylactic or therapeutic. Usually, the patient is a human, but in some diseases, such as mad cow disease, the patient can be a nonhuman mammal, such as a bovine. Treatment dosages need to be titrated to optimize safety and efficacy. The amount of immunogen depends on whether adjuvant is also administered, with higher dosages being required in the absence of adjuvant. The amount of an immunogen for administration sometimes varies from 1 μg-500 μg per patient and more usually from 5-500 μg per injection for human administration. Occasionally, a higher dose of 1-2 mg per injection is used. Typically about 10, 20, 50 or 100 μg is used for each human injection. The timing of injections can vary significantly from once a day, to once a year, to once a decade. On any given day that a dosage of immunogen is given, the dosage is greater than 1 μg/patient and usually greater than 10 μg/ patient if adjuvant is also administered, and greater than 10 μg/patient and usually greater than 100 μg/patient in the absence of adjuvant. A typical regimen consists of an immunization followed by booster injections at 6 weekly intervals. Another regimen consists of an immunization followed by booster injections 1, 2 and 12 months later. Another regimen entails an injection every two months for life. Alternatively, booster injections can be on an irregular basis as indicated by monitoring of immune response. For passive immunization with an antibody, the dosage ranges from about 0.0001 to 100 mg/kg, and more usually 0.01 to 5 mg/kg of the host body weight. Doses for nucleic acids encoding immunogens range from about 10 ng to 1 g, 100 ng to 100 mg, 1 μg to 10 mg, or 30-300 μg DNA per patient. Doses

25

for infectious viral vectors vary from 10-10^9, or more, virions per dose.

Agents for inducing an immune response can be administered by parenteral, topical, intravenous, oral, subcutaneous, intraperitoneal, intranasal or intramuscular means for prophylactic and/or therapeutic treatment. The most typical route of administration is subcutaneous although others can be equally effective. The next most common is intramuscular injection. This type of injection is most typically performed in the arm or leg muscles. Intravenous injections as well as intraperitoneal injections, intraarterial, intracranial, or intradermal injections are also effective in generating an immune response. In some methods, agents are injected directly into a particular tissue where deposits have accumulated.

Agents of the invention can optionally be administered in combination with other agents that are at least partly effective in treatment of amyloidogenic disease. In the case of Alzheimer's and Down's syndrome, in which amyloid deposits occur in the brain, agents of the invention can also be administered in conjunction with other agents that increase passage of the agents of the invention across the blood-brain barrier.

Immunogenic agents of the invention, such as peptides, are sometimes administered in combination with an adjuvant. A variety of adjuvants can be used in combination with a peptide, such as Aβ, to elicit an immune response. Preferred adjuvants augment the intrinsic response to an immunogen without causing conformational changes in the immunogen that affect the qualitative form of the response. Preferred adjuvants include alum, 3 De-O-acylated monophosphoryl lipid A (MPL) (see GB 2220211). QS21 is a triterpene glycoside or saponin isolated from the bark of the Quillaja Saponaria Molina tree found in South America (see Kensil et al., *in Vaccine Design: The Subunit and Ajuvant Approach* (eds. Powell & Newman, Plenum Press, NY, 1995); US Patent No. 5,057,540). Other adjuvants are oil in water emulsions (such as squalene or peanut oil), optionally in combination with immune

stimulants, such as monophosphoryl lipid A (see Stoute et al.,
N. Engl. J. Med. 336, 86-91 (1997)). Another adjuvant is CpG
(*Bioworld Today*, Nov. 15, 1998). Alternatively, Aβ can be
coupled to an adjuvant. For example, a lipopeptide version of
5   Aβ can be prepared by coupling palmitic acid or other lipids
directly to the N-terminus of Aβ as described for hepatitis B
antigen vaccination (Livingston, *J. Immunol.* 159, 1383-1392
(1997)). However, such coupling should not substantially
change the conformation of Aβ so as to affect the nature of
10   the immune response thereto. Adjuvants can be administered as
a component of a therapeutic composition with an active agent
or can be administered separately, before, concurrently with,
or after administration of the therapeutic agent.

A preferred class of adjuvants is aluminum salts (alum),
15   such as aluminum hydroxide, aluminum phosphate, aluminum
sulfate. Such adjuvants can be used with or without other
specific immunostimulating agents such as MPL or 3-DMP, QS21,
polymeric or monomeric amino acids such as polyglutamic acid
or polylysine. Another class of adjuvants is oil-in-water
20   emulsion formulations. Such adjuvants can be used with or
without other specific immunostimulating agents such as
muramyl peptides (e.g., N-acetylmuramyl-L-threonyl-D-
isoglutamine (thr-MDP), N-acetyl-normuramyl-L-alanyl-D-
isoglutamine (nor-MDP), N-acetylmuramyl-L-alanyl-D-
25   isoglutaminyl-L-alanine-2-(1'-2'dipalmitoyl-sn-glycero-3-
hydroxyphosphoryloxy)-ethylamine (MTP-PE), N-
acetylglucsaminyl-N-acetylmuramyl-L-Al-D-isoglu-L-Ala-
dipalmitoxy propylamide (DTP-DPP) theramide™), or other
bacterial cell wall components. Oil-in-water emulsions
30   include (a) MF59 (WO 90/14837), containing 5% Squalene, 0.5%
Tween 80, and 0.5% Span 85 (optionally containing various
amounts of MTP-PE) formulated into submicron particles using a
microfluidizer such as Model 110Y microfluidizer
(Microfluidics, Newton MA), (b) SAF, containing 10% Squalane,
35   0.4% Tween 80, 5% pluronic-blocked polymer L121, and thr-MDP,
either microfluidized into a submicron emulsion or vortexed to
generate a larger particle size emulsion, and (c) Ribi™
adjuvant system (RAS), (Ribi Immunochem, Hamilton, MT)

containing 2% squalene, 0.2% Tween 80, and one or more
bacterial cell wall components from the group consisting of
monophosphorylipid A (MPL), trehalose dimycolate (TDM), and
cell wall skeleton (CWS), preferably MPL + CWS (Detox™).

5   Another class of preferred adjuvants is saponin adjuvants,
such as Stimulon™ (QS21, Aquila, Worcester, MA) or particles
generated therefrom such as ISCOMs (immunostimulating
complexes) and ISCOMATRIX. Other adjuvants include Complete
Freund's Adjuvant (CFA) and Incomplete Freund's Adjuvant

10   (IFA). Other adjuvants include cytokines, such as
interleukins (IL-1,IL-2, and IL-12), macrophage colony
stimulating factor (M-CSF), tumor necrosis factor (TNF).

       An adjuvant can be administered with an immunogen as a
single composition, or can be administered before, concurrent

15   with or after administration of the immunogen. Immunogen and
adjuvant can be packaged and supplied in the same vial or can
be packaged in separate vials and mixed before use. Immunogen
and adjuvant are typically packaged with a label indicating
the intended therapeutic application. If immunogen and

20   adjuvant are packaged separately, the packaging typically
includes instructions for mixing before use. The choice of
an adjuvant and/or carrier depends on the stability of the
vaccine containing the adjuvant, the route of administration,
the dosing schedule, the efficacy of the adjuvant for the

25   species being vaccinated, and, in humans, a pharmaceutically
acceptable adjuvant is one that has been approved or is
approvable for human administration by pertinent regulatory
bodies. For example, Complete Freund's adjuvant is not
suitable for human administration. Alum, MPL and QS21 are

30   preferred. Optionally, two or more different adjuvants can be
used simultaneously. Preferred combinations include alum with
MPL, alum with QS21, MPL with QS21, and alum, QS21 and MPL
together. Also, Incomplete Freund's ajuvant can be used
(Chang et al., *Advanced Drug Delivery Reviews* 32, 173-186

35   (1998)), optionally in combination with any of alum, QS21, and
MPL and all combinations thereof.

       Agents of the invention are often administered as
pharmaceutical compositions comprising an active therapeutic

agent, *i.e.*, and a variety of other pharmaceutically acceptable components. *See Remington's Pharmaceutical Science* (15th ed., Mack Publishing Company, Easton, Pennsylvania, 1980). The preferred form depends on the intended mode of

5   administration and therapeutic application. The compositions can also include, depending on the formulation desired, pharmaceutically-acceptable, non-toxic carriers or diluents, which are defined as vehicles commonly used to formulate pharmaceutical compositions for animal or human

10   administration. The diluent is selected so as not to affect the biological activity of the combination. Examples of such diluents are distilled water, physiological phosphate-buffered saline, Ringer's solutions, dextrose solution, and Hank's solution. In addition, the pharmaceutical composition or

15   formulation may also include other carriers, adjuvants, or nontoxic, nontherapeutic, nonimmunogenic stabilizers and the like. However, some reagents suitable for administration to animals, such as Complete Freund's adjuvant are not typically included in compositions for human use.

20   Pharmaceutical compositions can also include large, slowly metabolized macromolecules such as proteins, polysaccharides, polylactic acids, polyglycolic acids and copolymers (such as latex functionalized sepharose, agarose, cellulose, and the like), polymeric amino acids, amino acid copolymers, and lipid

25   aggregates (such as oil droplets or liposomes). Additionally, these carriers can function as immunostimulating agents (i.e., adjuvants).

       For parenteral administration, agents of the invention can be administered as injectable dosages of a solution or

30   suspension of the substance in a physiologically acceptable diluent with a pharmaceutical carrier which can be a sterile liquid such as water oils, saline, glycerol, or ethanol. Additionally, auxiliary substances, such as wetting or emulsifying agents, surfactants, pH buffering substances and

35   the like can be present in compositions. Other components of pharmaceutical compositions are those of petroleum, animal, vegetable, or synthetic origin, for example, peanut oil, soybean oil, and mineral oil. In general, glycols such as

propylene glycol or polyethylene glycol are preferred liquid
carriers, particularly for injectable solutions.

Typically, compositions are prepared as injectables, either
as liquid solutions or suspensions; solid forms suitable for
solution in, or suspension in, liquid vehicles prior to
injection can also be prepared. The preparation also can be
emulsified or encapsulated in liposomes or micro particles
such as polylactide, polyglycolide, or copolymer for enhanced
adjuvant effect, as discussed above (see Langer, *Science* 249,
1527 (1990) and Hanes, *Advanced Drug Delivery Reviews* 28, 97-
119 (1997). The agents of this invention can be administered
in the form of a depot injection or implant preparation which
can be formulated in such a manner as to permit a sustained or
pulsatile release of the active ingredient.

Additional formulations suitable for other modes of
administration include oral, intranasal, and pulmonary
formulations, suppositories, and transdermal applications.

For suppositories, binders and carriers include, for
example, polyalkylene glycols or triglycerides; such
suppositories can be formed from mixtures containing the
active ingredient in the range of 0.5% to 10%, preferably 1%-
2%. Oral formulations include excipients, such as
pharmaceutical grades of mannitol, lactose, starch, magnesium
stearate, sodium saccharine, cellulose, and magnesium
carbonate. These compositions take the form of solutions,
suspensions, tablets, pills, capsules, sustained release
formulations or powders and contain 10%-95% of active
ingredient, preferably 25%-70%.

Topical application can result in transdermal or
intradermal delivery. Topical administration can be
facilitated by co-administration of the agent with cholera
toxin or detoxified derivatives or subunits thereof or other
similar bacterial toxins (See Glenn et al., *Nature* 391, 851
(1998)). Co-administration can be achieved by using the
components as a mixture or as linked molecules obtained by
chemical crosslinking or expression as a fusion protein.

Alternatively, transdermal delivery can be achieved using a
skin path or using transferosomes (Paul et al., *Eur. J.*

Immunol. 25, 3521-24 (1995); Cevc et al., *Biochem. Biophys.*
Acta 1368, 201-15 (1998)).

V.   <u>Methods of Diagnosis</u>

The invention provides methods of detecting an immune
response against Aβ peptide in a patient suffering from or
susceptible to Alzheimer's disease. The methods are
particularly useful for monitoring a course of treatment being
administered to a patient. The methods can be used to monitor
both therapeutic treatment on symptomatic patients and
prophylactic treatment on asymptomatic patients.

Some methods entail determining a baseline value of an
immune response in a patient before administering a dosage of
agent, and comparing this with a value for the immune response
after treatment. A significant increase (i.e., greater than
the typical margin of experimental error in repeat
measurements of the same sample, expressed as one standard
deviation from the mean of such measurments) in value of the
immune response signals a positive treatment outcome (i.e.,
that administration of the agent has achieved or augmented an
immune response). If the value for immune response does not
change significantly, or decreases, a negative treatment
outcome is indicated. In general, patients undergoing an
initial course of treatment with an agent are expected to show
an increase in immune response with successive dosages, which
eventually reaches a plateau. Administration of agent is
generally continued while the immune response is increasing.
Attainment of the plateau is an indicator that the
administered of treatment can be discontinued or reduced in
dosage or frequency.

In other methods, a control value (i.e., a mean and
standard deviation) of immune response is determined for a
control population. Typically the individuals in the control
population have not received prior treatment. Measured values
of immune response in a patient after administering a
therapeutic agent are then compared with the control value. A
significant increase relative to the control value (e.g.,
greater than one standard deviation from the mean) signals a

positive treatment outcome. A lack of significant increase or a decrease signals a negative treatment outcome. Administration of agent is generally continued while the immune response is increasing relative to the control value. As before, attainment of a plateau relative to control values in an indicator that the administration of treatment can be discontinued or reduced in dosage or frequency.

In other methods, a control value of immune response (e.g., a mean and standard deviation) is determined from a control population of individuals who have undergone treatment with a therapeutic agent and whose immune responses have plateaued in response to treatment. Measured values of immune response in a patient are compared with the control value. If the measured level in a patient is not significantly different (e.g., more than one standard deviation) from the control value, treatment can be discontinued. If the level in a patient is significantly below the control value, continued administration of agent is warranted. If the level in the patient persists below the control value, then a change in treatment regime, for example, use of a different adjuvant may be indicated.

In other methods, a patient who is not presently receiving treatment but has undergone a previous course of treatment is monitored for immune response to determine whether a resumption of treatment is required. The measured value of immune response in the patient can be compared with a value of immune response previously achieved in the patient after a previous course of treatment. A significant decrease relative to the previous measurement (i.e., greater than a typical margin of error in repeat measurements of the same sample) is an indication that treatment can be resumed. Alternatively, the value measured in patient can be compared with a control value (mean plus standard deviation) determined in population of patients after undergoing a course of treatment. Alternatively, the measured value in a patient can be compared with a control value in populations of prophylactically treated patients who remain free of symptoms of disease, or populations of therapeutically treated patients who show

amelioration of disease characteristics. In all of these cases, a significant decrease relative to the control level (i.e., more than a standard deviation) is an indicator that treatment should be resumed in a patient.

The tissue sample for analysis is typically blood, plasma, serum, mucus or cerebral spinal fluid from the patient. The sample is analyzed for indicia of an immune response to any form of Aβ peptide, typically Aβ42. The immune response can be determined from the presence of, e.g., antibodies or T-cells that specifically bind to Aβ peptide. ELISA methods of detecting antibodies specific to Aβ are described in the Examples section. Methods of detecting reactive T-cells have been described above (see Definitions).

The invention further provides diagnostic kits for performing the diagnostic methods described above. Typically, such kits contain an agent that specifically binds to antibodies to Aβ or reacts with T-cells specific for Aβ. The kit can also include a label. For detection of antibodies to Aβ, the label is typically in the form of labelled anti-idiotypic antibodies. For detection of antibodies, the agent can be supplied prebound to a solid phase, such as to the wells of a microtiter dish. For detection of reactive T-cells, the label can be supplied as ^{3}H-thymidine to measure a proliferative response. Kits also typically contain labelling providing directions for use of the kit. The labelling may also include a chart or other correspondence regime correlating levels of measured label with levels of antibodies to Aβ or T-cells reactive with Aβ. The term labelling refers to any written or recorded material that is attached to, or otherwise accompanies a kit at any time during its manufacture, transport, sale or use. For example, the term labelling encompasses advertising leaflets and brochures, packaging materials, instructions, audio or video cassettes, computer discs, as well as writing imprinted directly on kits.

EXAMPLES

I. Prophylactic Efficacy of Aβ Against AD

These examples describe administration of Aβ42 peptide to transgenic mice overexpressing APP with a mutation at position 717 (APP$_{717V \rightarrow F}$) that predisposes them to develop Alzheimer's-like neuropathology. Production and characteristics of these

5   mice (PDAPP mice) is described in Games et al., *Nature, supra.* These animals, in their heterozygote form, begin to deposit Aβ at six months of age forward. By fifteen months of age they exhibit levels of Aβ deposition equivalent to that seen in Alzheimer's disease. PDAPP mice were injected with aggregated

10   Aβ_{42} (aggregated Aβ_{42}) or phosphate buffered saline. Aggregated Aβ_{42} was chosen because of its ability to induce antibodies to multiple epitopes of Aβ.

A. Methods

   1. Source of Mice

   Thirty PDAPP heterogenic female mice were randomly divided into the following groups: 10 mice to be injected with aggregated $A\beta_{42}$ (one died in transit), 5 mice to be injected with PBS/adjuvant or PBS, and 10 uninjected controls. Five mice were injected with serum amyloid protein (SAP).

   2. Preparation of Immunogens

   Preparation of aggregated $A\beta_{42}$: two milligrams of $A\beta_{42}$ (US Peptides Inc, lot K-42-12) was dissolved in 0.9 ml water and made up to 1 ml by adding 0.1 ml 10 x PBS. This was vortexed and allowed to incubate overnight 37° C, under which conditions the peptide aggregated. Any unused $A\beta$ was stored as a dry lyophilized powder at -20° C until the next injection.

   3. Preparation of Injections

   100 μg of aggregated $A\beta_{42}$ in PBS per mouse was emulsified 1:1 with Complete Freund's adjuvant (CFA) in a final volume of 400 μl emulsion for the first immunization, followed by a boost of the same amount of immunogen in Incomplete Freund's adjuvant (IFA) at 2 weeks. Two additional doses in IFA were given at monthly intervals. The subsequent immunizations were done at monthly intervals in 500 μl of PBS. Injections were delivered intraperitoneally (i.p.).

   PBS injections followed the same schedule and mice were injected with a 1:1 mix of PBS/ Adjuvant at 400 μl per mouse, or 500 μl of PBS per mouse. SAP injections likewise followed the same schedule using a dose of 100 μg per injection.

   4. Titration of Mouse Bleeds, Tissue Preparation
       and Immunohistochemistry

   The above methods are described infra in General Materials and Methods.

B. Results

   PDAPP mice were injected with either aggregated $A\beta_{42}$ (aggregated $A\beta_{42}$), SAP peptides, or phosphate buffered saline.

A group of PDAPP mice were also left as uninjected, positive controls. The titers of the mice to aggregated $A\beta_{42}$ were monitored every other month from the fourth boost until the mice were one year of age. Mice were sacrificed at 13 months.

5   At all time points examined, eight of the nine aggregated $A\beta_{42}$ mice developed a high antibody titer, which remained high throughout the series of injections (titers greater than 1/10000). The ninth mouse had a low, but measurable titer of approximately 1/1000 (Figure 1, Table 1). SAPP-injected mice

10 had titers of 1:1,000 to 1:30,000 for this immunogen with only a single mice exceeding 1:10,0000.

The PBS-treated mice were titered against aggregated $A\beta_{42}$ at six, ten and twelve months. At a 1/100 dilution the PBS mice when titered against aggregated $A\beta_{42}$ only exceeded 4

15 times background at one data point, otherwise, they were less than 4 times background at all time points (Table 1). The SAP-specific response was negligible at these time points with all titers less than 300.

Seven out of the nine mice in the aggregated $A\beta 1-42$ group

20 had no detectable amyloid in their brains. In contrast, brain tissue from mice in the SAP and PBS groups contained numerous 3D6-positive amyloid deposits in the hippocampus, as well as in the frontal and cingulate cortices. The pattern of deposition was similar to that of untreated controls, with

25 characteristic involvement of vulnerable subregions, such as the outer molecular layer of the hippocampal dentate gyrus. One mouse from the $A\beta$ 1-42-injected group had a greatly reduced amyloid burden, confined to the hippocampus. An isolated plaque was identified in another $A\beta$ 1-42-treated

30 mouse.

Quantitative image analyses of the amyloid burden in the hippocampus verified the dramatic reduction achieved in the AN1792-treated animals (Fig. 2). The median values of the amyloid burden for the PBS group (2.22%), and for the

35 untreated control group (2.65%) were significantly greater than for those immunized with AN1792 (0.00%, p=0.0005). In contrast, the median value for the group immunized with SAP peptides (SAPP) was 5.74%. Brain tissue from the untreated,

control mice contained numerous Aβ amyloid deposits visualized with the Aβ-specific monoclonal antibody (mAb) 3D6 in the hippocampus, as well as in the retrosplenial cortex. A similar pattern of amyloid deposition was also seen in mice immunized with SAPP or PBS (Fig. 2). In addition, in these latter three groups there was a characteristic involvement of vulnerable subregions of the brain classically seen in AD, such as the outer molecular layer of the hippocampal dentate gyrus, in all three of these groups.

The brains that contained no Aβ deposits were also devoid of neuritic plaques that are typically visualized in PDAPP mice with the human APP antibody 8E5. All of brains from the remaining groups (SAP-injected, PBS and uninjected mice) had numerous neuritic plaques typical of untreated PDAPP mice. A small number of neuritic plaques were present in one mouse treated with AN1792, and a single cluster of dystrophic neurites was found in a second mouse treated with AN1792. Image analyses of the hippocampus, and shown in Fig. 3, demonstrated the virtual elimination of dystrophic neurites in AN1792-treated mice (median 0.00%) compared to the PBS recipients (median 0.28%, p = 0.0005).

Astrocytosis characteristic of plaque-associated inflammation was also absent in the brains of the Aβ1-42 injected group. The brains from the mice in the other groups contained abundant and clustered GFAP-positive astrocytes typical of Aβ plaque-associated gliosis. A subset of the GFAP-reacted slides were counter-stained with Thioflavin S to localize the Aβ deposits. The GFAP-positive astrocytes were associated with Aβ plaques in the SAP, PBS and untreated controls. No such association was found in the plaque-negative Aβ1-42 treated mice, while minimal plaque-associated gliosis was identified in one mouse treated with AN1792.

Image analyses, shown in Fig. 4 for the retrosplenial cortex, verified that the reduction in astrocytosis was significant with a median value of 1.56% for those treated with AN1792 versus median values greater than 6% for groups immunized with SAP peptides, PBS or untreated (p=0.0017)

Evidence from a subset of the Aβ 1-42- and PBS-injected
mice indicated plaque-associated MHC II immunoreactivity was
absent in the Aβ1-42 injected mice, consistent with lack of an
Aβ-related inflammatory response.

5   Sections of the mouse brains were also reacted with a mAβ
specific for MAC-1, a cell surface protein. MAC-1 (CD11b) is
an integrin family member and exists as a heterodimer with
CD18. The CD11b/CD18 complex is present on monocytes,
macrophages, neutrophils and natural killer cells (Mak and
10   Simard). The resident MAC-1-reactive cell type in the brain
is likely to be microglia based on similar phenotypic
morphology in MAC-1 immunoreacted sections. Plaque-associated
MAC-1 labeling was lower in the brains of mice treated with
AN1792 compared to the PBS control group, a finding consistent
15   with the lack of an Aβ-induced inflammatory response.

C.   Conclusion
     The lack of Aβ plaques and reactive neuronal and gliotic
changes in the brains of the Aβ1-42-injected mice indicate
that no or extremely little amyloid was deposited in their
20   brains, and pathological consequences, such as gliosis and
neuritic pathology, were absent. PDAPP mice treated with
Aβ1-42 show essentially the same lack of pathology as control
nontransgenic mice. Therefore, Aβ1-42 injections are highly
effective in the prevention of deposition or clearance of
25   human Aβ from brain tissue, and elimination of subsequent
neuronal and inflammatory degenerative changes. Thus,
administration of Aβ peptide has therapeutic benefit in
prevention of AD.

II. Dose Response Study
30   Groups of five-week old, female Swiss Webster mice (N=6 per
group) were immunized with 300, 100, 33, 11, 3.7, 1.2, 0.4, or
0.13 ug of Aβ formulated in CFA/IFA administered
intraperitoneally. Three doses were given at biweekly
intervals followed by a fourth dose one month later. The
35   first dose was emulsified with CFA and the remaining doses
were emulsified with IFA. Animals were bled 4-7 days

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following each immunization starting after the second dose
for measurement of antibody titers. Animals in a subset of
three groups, those immunized with 11, 33, or 300 μg of
antigen, were additionally bled at approximately monthly

5   intervals for four months following the fourth immunization to
monitor the decay of the antibody response across a range of
vaccine doses. These animals received a final fifth
immunization at seven months after study initiation. They
were sacrificed one week later to measure antibody responses

10   to AN1792 and to perform toxicological analyses.

A declining dose response was observed from 300 to 3.7 μg
with no response at the two lowest doses. Mean antibody
titers are about 1:1000 after 3 doses and about 1:10,000 after
4 doses of 11-300 μg of antigen (see Fig. 5).

15   Antibody titers rose dramatically for all but the lowest
dose group following the third immunization with increases in
GMTs ranging from 5- to 25-fold. Low antibody responses were
then detectable for even the 0.4 μg recipients. The 1.2 and
3.7 μg groups had comparable titers with GMTs of about 1000

20   and the highest four doses clustered together with GMTs of
about 25,000, with the exception of the 33 μg dose group with
a lower GMT of 3000. Following the fourth immunization, the
titer increase was more modest for most groups. There was a
clear dose response across the lower antigen dose groups from

25   0.14 μg to 11 μg ranging from no detectable antibody for
recipients of 0.14 μg to a GMT of 36,000 for recipients of
11 μg. Again, titers for the four highest dose groups of 11
to 300 μg clustered together. Thus following two
immunizations, the antibody titer was dependent on the antigen

30   dose across the broad range from 0.4 to 300 μg. By the third
immunization, titers of the highest four doses were all
comparable and they remained at a plateau after an additional
immunization.

One month following the fourth immunization, titers were 2-

35   to 3-fold higher in the 300 μg group than those measured from
blood drawn five days following the immunization (Fig. 6).
This observation suggests that the peak anamnestic antibody
response occurred later than 5 days post-immunization. A more

modest (50%) increase was seen at this time in the 33 μg
group. In the 300 μg dose group at two months following the
last dose, GMTs declined steeply by about 70%. After another
month, the decline was less steep at 45% (100 μg) and about

5   14% for the 33 and 11 μg doses. Thus, the rate of decline in
circulating antibody titers following cessation of
immunization appears to be biphasic with a steep decline the
first month following peak response followed by a more modest
rate of decrease thereafter.

10   The antibody titers and the kinetics of the response of
these Swiss Webster mice are similar to those of young
heterozygous PDAPP transgenic mice immunized in a parallel
manner. Dosages effective to induce an immune response in
humans are typically similar to dosages effective in mice.

15   III. Screen For Therapeutic Efficacy Against Established AD
This assay is designed to test immunogenic agents for
activity in arresting or reversing neuropathological
characteristics of AD in aged animals. Immunizations with 42
amino acid long Aβ (AN1792) were begun at a timepoint when

20   amyloid plaques are already present in the brains of the PDAPP
mice.
Over the timecourse used in this study, untreated PDAPP
mice develop a number of neurodegenerative changes that
resemble those found in AD (Games et al., *supra* and Johnson-

25   Wood et al., *Proc. Natl. Acad. Sci. USA* 94, 1550-1555 (1997)).
The deposition of Aβ into amyloid plaques is associated with a
degenerative neuronal response consisting of aberrant axonal
and dendritic elements, called dystrophic neurites. Amyloid
deposits that are surrounded by and contain dystrophic

30   neurites called neuritic plaques. In both AD and the PDAPP
mouse, dystrophic neurites have a distinctive globular
structure, are immunoreactive with a panel of antibodies
recognizing APP and cytoskeletal components, and display
complex subcellular degenerative changes at the

35   ultrastructural level. These characteristics allow for
disease-relevant, selective and reproducible measurements of
neuritic plaque formation in the PDAPP brains. The dystrophic

neuronal component of PDAPP neuritic plaques is easily
visualized with an antibody specific for human APP (mAβ 8E5),
and is readily measurable by computer-assisted image analysis.
Therefore, in addition to measuring the effects of AN1792 on
5   amyloid plaque formation, we monitored the effects of this
treatment on the development of neuritic dystrophy.

Astrocytes and microglia are non-neuronal cells that
respond to and reflect the degree of neuronal injury. GFAP-
positive astrocytes and MHC II-positive microglia are commonly
10   observed in AD, and their activation increases with the
severity of the disease. Therefore, we also monitored the
development of reactive astrocytosis and microgliosis in the
AN1792-treated mice.

A.   Materials and Methods
15   Forty-eight, heterozygous female PDAPP mice, 11 to 11.5
months of age, obtained from Charles River, were randomly
divided into two groups: 24 mice to be immunized with 100 μg
of AN1792 and 24 mice to be immunized with PBS, each combined
with Freund's adjuvant. The AN1792 and PBS groups were again
20   divided when they reached ˜15 months of age. At 15 months of
age approximately half of each group of the AN1792- and PBS-
treated animals were euthanized (n=10 and 9, respectively),
the remainder continued to receive immunizations until
termination at ˜18 months (n=9 and 12, respectively). A total
25   of 8 animals (5 AN1792, 3 PBS) died during the study. In
addition to the immunized animals, one-year old (n=10), 15-
month old (n=10) and 18-month old (n=10) untreated PDAPP mice
were included for comparison in the ELISAs to measure aβ and
APP levels in the brain; the one-year old animals were also
30   included in the immunohistochemical analyses.

Methodology was as in Example 1 unless otherwise indicated.
US Peptides lot 12 and California Peptides lot ME0339 of
AN1792 were used to prepare the antigen for the six
immunizations administered prior to the 15-month timepoint.
35   California Peptides lots ME0339 and ME0439 were used for the
three additional immunizations administered between 15 and 18
months.

For immunizations, 100 μg of AN1792 in 200 μl PBS or PBS alone was emulsified 1:1 (vol:vol) with Complete Freund's adjuvant (CFA) or Incomplete Freund's adjuvant (IFA) or PBS in a final volume of 400 μl. The first immunization was

5 delivered with CFA as adjuvant, the next four doses were given with IFA and the final four doses with PBS alone without added adjuvant. A total of nine immunizations were given over the seven-month period on a two-week schedule for the first three doses followed by a four-week interval for the remaining

10 injections. The four-month treatment group, euthanized at 15 months of age, received only the first 6 immunizations.

B.   Results

     1.   Effects of AN1792 Treatment on Amyloid Burden

     The results of AN1792 treatment on cortical amyloid burden

15 determined by quantitative image analysis are shown in Fig. 7. The median value of cortical amyloid burden was 0.28% in a group of untreated 12-month old PDAPP mice, a value representative of the plaque load in mice at the study's initiation. At 18 months, the amyloid burden increased over

20 17-fold to 4.87% in PBS-treated mice, while AN1792-treated mice had a greatly reduced amyloid burden of only 0.01%, notably less than the 12-month untreated and both the 15- and 18-month PBS-treated groups. The amyloid burden was significantly reduced in the AN1792 recipients at both 15 (96%

25 reduction; p=0.003) and 18 (>99% reduction; p=0.0002) months. Typically, cortical amyloid deposition in PDAPP mice initiates in the frontal and retrosplenial cortices (RSC) and progresses in a ventral-lateral direction to involve the temporal and entorhinal cortices (EC). Little or no amyloid

30 was found in the EC of 12 month-old mice, the approximate age at which AN1792 was first administered. After 4 months of AN1792 treatment, amyloid deposition was greatly diminished in the RSC, and the progressive involvement of the EC was entirely eliminated by AN1792 treatment. The latter

35 observation showed that AN1792 completely halted the progression of amyloid that would normally invade the temporal

42

and ventral cortices, as well as arrested or possibly reversed deposition in the RSC.

The profound effects of AN1792 treatment on developing cortical amyloid burden in the PDAPP mice are further demonstrated by the 18-month group, which had been treated for seven months. A near complete absence of cortical amyloid was found in the AN1792-treated mouse, with a total lack of diffuse plaques, as well as a reduction in compacted deposits.

2. AN1792 Treatment-associated Cellular and Morphological Changes

A population of Aβ-positive cells was found in brain regions that typically contain amyloid deposits. Remarkably, in several brains from AN1792 recipients, very few or no extracellular cortical amyloid plaques were found. Most of the Aβ immunoreactivity appeared to be contained within cells with large lobular or clumped soma. Phenotypically, these cells resembled activated microglia or monocytes. They were immunoreactive with antibodies recognizing ligands expressed by activated monocytes and microglia (MHC II and CD11b) and were occasionally associated with the wall or lumen of blood vessels. Comparison of near-adjacent sections labeled with Aβ and MHC II-specific antibodies revealed that similar patterns of these cells were recognized by both classes of antibodies. Detailed examination of the AN1792-treated brains revealed that the MHC II-positive cells were restricted to the vicinity of the limited amyloid remaining in these animals. Under the fixation conditions employed, the cells were not immunoreactive with antibodies that recognize T cell (CD3, CD3e) or B cell (CD45RA, CD45RB) ligands or leukocyte common antigen (CD45), but were reactive with an antibody recognizing leukosialin (CD43) which cross-reacts with monocytes. No such cells were found in any of the PBS-treated mice.

PDAPP mice invariably develop heavy amyloid deposition in the outer molecular layer of the hippocampal dentate gyrus. The deposition forms a distinct streak within the perforant pathway, a subregion that classically contains amyloid plaques in AD. The characteristic appearance of these deposits in PBS-treated mice resembled that previously characterized in

untreated PDAPP mice. The amyloid deposition consisted of
both diffuse and compacted plaques in a continuous band. In
contrast, in a number of brains from AN1792-treated mice this
pattern was drastically altered. The hippocampal amyloid
5   deposition no longer contained diffuse amyloid, and the banded
pattern was completely disrupted. Instead, a number of
unusual punctate structures were present that are reactive
with anti-Aβ antibodies, several of which appeared to be
amyloid-containing cells.

10   MHC II-positive cells were frequently observed in the
vicinity of extracellular amyloid in AN1792-treated animals.
The pattern of association of Aβ-positive cells with amyloid
was very similar in several brains from AN1792-treated mice.
The distribution of these monocytic cells was restricted to
15   the proximity of the deposited amyloid and was entirely absent
from other brain regions devoid of Aβ plaques.

         Quantitative image analysis of MHC II and MAC I-labeled
sections revealed a trend towards increased immunoreactivity
in the RSC and hippocampus of AN1792-treated mice compared to
20   the PBS group which reached significance with the measure of
MAC 1 reactivity in hippocampus.

         These results are indicative of active, cell-mediated
removal of amyloid in plaque-bearing brain regions.

    3.   AN1792 Effects on Aβ Levels: ELISA Determinations
25   . (a) Cortical Levels
         In untreated PDAPP mice, the median level of total Aβ in
the cortex at 12 months was 1,600 ng/g, which increased to
8,700 ng/g by 15 months (Table 2). At 18 months the value was
22,000 ng/g, an increase of over 10-fold during the time
30   course of the experiment. PBS-treated animals had 8,600 ng/g
total Aβ at 15 months which increased to 19,000 ng/g at 18
months. In contrast, AN1792-treated animals had 81% less
total Aβ at 15 months (1,600 ng/g) than the PBS-immunized
group. Significantly less (p=0.0001) total Aβ (5,200 ng/g)
35   was found at 18 months when the AN1792 and PBS groups were
compared (Table 2), representing a 72% reduction in the Aβ
that would otherwise be present. Similar results were

obtained when cortical levels of Aβ42 were compared, namely
that the AN1792-treated group contained much less Aβ42, but in
this case the differences between the AN1792 and PBS groups
were significant at both 15 months (p=0.04) and 18 months
5   (p=0.0001, Table 2).

Table 2: Median Aβ Levels (ng/g) in Cortex

	UNTREATED			PBS			AN1792		
Age	Total Aβ	Aβ42	(n)	Total Aβ	Aβ42	(n)	Total	Aβ42	(n)
12	1,600	1,300	(10)						
15	8,700	8,300	(10)	8,600	7,200	(9)	1,600	1,300*	(10)
18	22,200	18,500	(10)	19,000	15,900	(12)	5,200**	4,000**	(9)

* p = 0.0412

** p = 0.0001

(b)   Hippocampal Levels

In untreated PDAPP mice, median hippocampal levels of total
Aβ at twelve months of age were 15,000 ng/g which increased to
51,000 ng/g at 15 months and further to 81,000 ng/g at 18
months (Table 3). Similarly, PBS immunized mice showed values
of 40,000 ng/g and 65,000 ng/g at 15 months and 18 months,
20   respectively. AN1792 immunized animals exhibited less total
Aβ, specifically 25,000 ng/g and 51,000 ng/g at the
respective 15-month and 18-month timepoints. The 18-month
AN1792-treated group value was significantly lower than that
of the PBS treated group (p= 0.0105; Table 3). Measurement of
25   Aβ42 gave the same pattern of results, namely that levels in
the AN1792-treated group were significantly lower than in the
PBS group (39,000 ng/g vs. 57,000 ng/g, respectively;
p=0.0022) at the 18-month evaluation (Table 3).

45

Table 3:   Median Aβ Levels (ng/g) in Hippocampus

	UNTREATED			PBS			AN1792		
Age	Total Aβ	Aβ42	(n)	Total Aβ	Aβ42	(n)	Total	Aβ42	(n)
12	15,500	11,100	(10)						
15	51,500	44,400	(10)	40,100	35,700	(9)	24,500	22,100	(10)
18	80,800	64,200	(10)	65,400	57,100	(12)	50,900*	38,900**	(9)

* p = 0.0105

** p = 0.0022

(c)   Cerebellar Levels

In 12-month untreated PDAPP mice, the median cerebellar
level of total Aβ was 15 ng/g (Table 4). At 15 months, this
median increased to 28 ng/g and by 18 months had risen to 35
ng/g. PBS-treated animals displayed median total Aβ values of
21 ng/g at 15 months and 43 ng/g at 18 months. AN1792-treated
animals were found to have 22 ng/g total Aβ at 15 months and
significantly less (p=0.002) total Aβ at 18 months (25 ng/g)
than the corresponding PBS group (Table 4).

Table 4:   Median Aβ Levels (ng/g) in Cerebellum

	UNTREATED		PBS		AN1792	
Age (months)	Total Aβ	(n)	Total Aβ	(n)	Total Aβ	(n)
12	15.6	(10)				
15	27.7	(10)	20.8	(9)	21.7	(10)
18	35.0	(10)	43.1	(12)	24.8*	(9)

* p = 0.0018

4.   Effects of AN1792 Treatment on APP Levels

APP-α and the full-length APP molecule both contain all or
part of the Aβ sequence and thus could be potentially impacted
by the generation of an AN1792-directed immune response. In
studies to date, a slight increase in APP levels has been
noted as neuropathology increases in the PDAPP mouse. In the
cortex, levels of either APP-α/FL (full length) or APP-α were
essentially unchanged by treatment with the exception that
APP-α was reduced by 19% at the 18-month timepoint in the
AN1792-treated vs. the PBS-treated group. The 18-month
AN1792-treated APP values were not significantly different

from values of the 12-month and 15-month untreated and 15-
month PBS groups. In all cases the APP values remained within
the ranges that are normally found in PDAPP mice.

5.   Effects of AN1792 Treatment on Neurodegenerative and
      Gliotic Pathology

Neuritic plaque burden was significantly reduced in the
frontal cortex of AN1792-treated mice compared to the PBS
group at both 15 (84%; p=0.03) and 18 (55%; p=0.01) months of
age (Fig. 8). The median value of the neuritic plaque burden
increased from 0.32% to 0.49% in the PBS group between 15 and
18 months of age. This contrasted with the greatly reduced
development of neuritic plaques in the AN1792 group, with
median neuritic plaque burden values of 0.05% and 0.22%, in
the 15 and 18 month groups, respectively.

Immunizations with AN1792 seemed well tolerated and
reactive astrocytosis was also significantly reduced in the
RSC of AN1792-treated mice when compared to the PBS group at
both 15 (56%; p=0.011) and 18 (39%; p=0.028) months of age
(Fig. 9). Median values of the percent of astrocytosis in
the PBS group increased between 15 and 18 months from 4.26% to
5.21%. AN1792-treatment suppressed the development of
astrocytosis at both time points to 1.89% and 3.2%,
respectively. This suggests the neuropil was not being
damaged by the clearance process.

6. Antibody Responses

As described above, eleven-month old, heterozygous PDAPP
mice (N=24) received a series of 5 immunizations of 100 μg of
AN1792 emulsified with Freund's adjuvant and administered
intraperitoneally at weeks 0, 2, 4, 8, and 12, and a sixth
immunization with PBS alone (no Freund's adjuvant) at week 16.
As a negative control, a parallel set of 24 age-matched
transgenic mice received immunizations of PBS emulsified with
the same adjuvants and delivered on the same schedule.
Animals were bled within three to seven days following each
immunization starting after the second dose. Antibody
responses to AN1792 were measured by ELISA. Geometric mean
titers (GMT) for the animals that were immunized with AN1792

were approximately 1,900, 7,600, and 45,000 following the
second, third and last (sixth) doses respectively. No Aβ-
specific antibody was measured in control animals following
the sixth immunization.

Approximately one-half of the animals were treated for an
additional three months, receiving immunizations at about 20,
24 and 27 weeks. Each of these doses was delivered in PBS
vehicle alone without Freund's adjuvant. Mean antibody titers
remained unchanged over this time period. In fact, antibody
titers appeared to remain stable from the fourth to the eighth
bleed corresponding to a period covering the fifth to the
ninth injections.

To determine if the Aβ-specific antibodies elicited by
immunization that were detected in the sera of AN1792-treated
mice were also associated with deposited brain amyloid, a
subset of sections from the AN1792- and PBS-treated mice were
reacted with an antibody specific for mouse IgG. In contrast
to the PBS group, Aβ plaques in AN1792-treated brains were
coated with endogenous IgG. This difference between the two
groups was seen in both 15-and 18-month groups. Particularly
striking was the lack of labeling in the PBS group, despite
the presence of a heavy amyloid burden in these mice. These
results show that immunization with a synthetic Aβ protein
generates antibodies that recognize and bind *in vivo* to the Aβ
in amyloid plaques.

7. Cellular-Mediated Immune Responses

Spleens were removed from nine AN1792-immunized and 12
PBS-immunized 18-month old PDAPP mice 7 days after the ninth
immunization. Splenocytes were isolated and cultured for 72 h
in the presence of Aβ40, Aβ42, or Aβ40-1 (reverse order
protein). The mitogen Con A served as a positive control.
Optimum responses were obtained with >1.7 μM protein. Cells
from all nine AN1792-treated animals proliferated in response
to either Aβ1-40 or Aβ1-42 protein, with equal levels of
incorporation for both proteins (Fig. 10, Upper Panel). There
was no response to the Aβ40-1 reverse protein. Cells from

control animals did not respond to any of the Aβ proteins (Fig. 10, Lower Panel).

C. Conclusion

The results of this study show that AN1792 immunization of PDAPP mice possessing existing amyloid deposits slows and prevents progressive amyloid deposition and retard consequential neuropathological changes in the aged PDAPP mouse brain. Immunizations with AN1792 essentially halted amyloid developing in structures that would normally succumb to amyloidosis. Thus, administration of Aβ peptide has therapeutic benefit in the treatment of AD.

IV. Screen of Aβ Fragments

100 PDAPP mice age 9-11 months are immunized with 9 different regions of APP and Aβ to determine which epitopes convey the response. The 9 different immunogens and one control are injected i.p. as described above. The immunogens include four human Aβ peptide conjugates 1-12, 13-28, 32-42, 1-5, all coupled to sheep anti-mouse IgG via a cystine link; an APP polypeptide aa 592-695, aggregated human Aβ 1-40, and aggregated human Aβ 25-35, and aggregated rodent Aβ42. Aggregated Aβ42 and PBS are used as controls. Ten mice are used per treatment group. Titers are monitored as above and mice are euthanized at the end of 4 months of injections. Histochemistry, Aβ levels, and toxicology are determined post mortem.

A. Materials and Methods
1. Preparation of Immunogens

Preparation of coupled Aβ peptides: four human Aβ peptide conjugates (amino acid residues 1-5, 1-12, 13-28, and 33-42, each conjugated to sheep anti-mouse IgG) were prepared by coupling through an artificial cysteine added to the Aβ peptide using the crosslinking reagent sulfo-EMCS. The Aβ peptide derivatives were synthesized with the following final amino acid sequences. In each case, the location of the inserted cysteine residue is indicated by underlining. The

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Aβ13-28 peptide derivative also had two glycine residues added prior to the carboxyl terminal cysteine as indicated.

Aβ1-12 peptide	NH₂-DAEFRHDSGYEV<u>C</u> COOH
Aβ1-5 peptide	NH₂-DAEFR<u>C</u> COOH
Aβ33-42 peptide	NH₂-<u>C-amino-heptanoic acid</u>-GLMVGGVVIA COOH
Aβ13-28 peptide	Ac-NH-HHQKLVFFAEDVGSNK<u>GGC</u>-COOH

To prepare for the coupling reaction, ten mg of sheep anti-mouse IgG (Jackson ImmunoResearch Laboratories) was dialyzed overnight against 10 mM sodium borate buffer, pH 8.5. The dialyzed antibody was then concentrated to a volume of 2 mL using an Amicon Centriprep tube. Ten mg sulfo-EMCS [N (ε-maleimidocuproyloxy) succinimide] (Molecular Sciences Co.) was dissolved in one mL deionized water. A 40-fold molar excess of sulfo-EMCS was added dropwise with stirring to the sheep anti-mouse IgG and then the solution was stirred for an additional ten min. The activated sheep anti-mouse IgG was purified and buffer exchanged by passage over a 10 mL gel filtration column (Pierce Presto Column, obtained from Pierce Chemicals) equilibrated with 0.1 M NaPO₄, 5 mM EDTA, pH 6.5. Antibody containing fractions, identified by absorbance at 280 nm, were pooled and diluted to a concentration of approximately 1 mg/mL, using 1.4 mg per OD as the extinction coefficient. A 40-fold molar excess of Aβ peptide was dissolved in 20 mL of 10 mM NaPO₄, pH 8.0, with the exception of the Aβ33-42 peptide for which 10 mg was first dissolved in 0.5 mL of DMSO and then diluted to 20 mL with the 10 mM NaPO₄ buffer. The peptide solutions were each added to 10 mL of activated sheep anti-mouse IgG and rocked at room temperature for 4 hr. The resulting conjugates were concentrated to a final volume of less than 10 mL using an Amicon Centriprep tube and then dialyzed against PBS to buffer exchange the buffer and remove free peptide. The conjugates were passed through 0.22 μ-pore size filters for sterilization and then aliquoted into fractions of 1 mg and stored frozen at -20°C. The concentrations of the conjugates were determined using the BCA protein assay (Pierce Chemicals) with horse IgG for the

standard curve. Conjugation was documented by the molecular weight increase of the conjugated peptides relative to that of the activated sheep anti-mouse IgG. The Aβ 1-5 sheep anti-mouse conjugate was a pool of two conjugations, the rest were

5   from a single preparation.

2.   Preparation of aggregated Aβ peptides

Human 1-40 (AN1528; California Peptides Inc., Lot ME0541), human 1-42 (AN1792; California Peptides Inc., Lots ME0339 and ME0439), human 25-35, and rodent 1-42 (California Peptides

10   Inc., Lot ME0218) peptides were freshly solubilized for the preparation of each set of injections from lyophilized powders that had been stored desiccated at -20°C. For this purpose, two mg of peptide were added to 0.9 ml of deionized water and the mixture was vortexed to generate a relatively uniform

15   solution or suspension. Of the four, AN1528 was the only peptide soluble at this step. A 100 μl aliquot of 10X PBS (1X PBS: 0.15 M NaCl, 0.01 M sodium phosphate, pH 7.5) was then added at which point AN1528 began to precipitate. The suspension was vortexed again and incubated overnight at 37°C

20   for use the next day.

Preparation of the pBx6 protein: An expression plasmid encoding pBx6, a fusion protein consisting of the 100-amino acid bacteriophage MS-2 polymerase N-terminal leader sequence followed by amino acids 592-695 of APP (βAPP) was constructed

25   as described by Oltersdorf et al., *J. Biol. Chem.* 265, 4492-4497 (1990). The plasmid was transfected into *E. coli* and the protein was expressed after induction of the promoter. The bacteria were lysed in 8M urea and pBx6 was partially purified by preparative SDS PAGE. Fractions containing pBx6 were

30   identified by Western blot using a rabbit anti-pBx6 polyclonal antibody, pooled, concentrated using an Amicon Centriprep tube and dialysed against PBS. The purity of the preparation, estimated by Coomassie Blue stained SDS PAGE, was approximately 5 to 10%.

B. Results and Discussion

1. Study Design

One hundred male and female, nine- to eleven-month old heterozygous PDAPP transgenic mice were obtained from Charles River Laboratory and Taconic Laboratory. The mice were sorted into ten groups to be immunized with different regions of Aβ or APP combined with Freund's adjuvant. Animals were distributed to match the gender, age, parentage and source of the animals within the groups as closely as possible. The immunogens included four Aβ peptides derived from the human sequence, 1-5, 1-12, 13-28, and 33-42, each conjugated to sheep anti-mouse IgG; four aggregated Aβ peptides, human 1-40 (AN1528), human 1-42 (AN1792), human 25-35, and rodent 1-42; and a fusion polypeptide, designated as pBx6, containing APP amino acid residues 592-695. A tenth group was immunized with PBS combined with adjuvant as a control.

For each immunization, 100 μg of each Aβ peptide in 200 μl PBS or 200 μg of the APP derivative pBx6 in the same volume of PBS or PBS alone was emulsified 1:1 (vol:vol) with Complete Freund's adjuvant (CFA) in a final volume of 400 μl for the first immunization, followed by a boost of the same amount of immunogen in Incomplete Freund's adjuvant (IFA) for the subsequent four doses and with PBS for the final dose. Immunizations were delivered intraperitoneally on a biweekly schedule for the first three doses, then on a monthly schedule thereafter. Animals were bled four to seven days following each immunization starting after the second dose for the measurement of antibody titers. Animals were euthanized approximately one week after the final dose.

2. Aβ and APP Levels in the Brain

Following about four months of immunization with the various Aβ peptides or the APP derivative, brains were removed from saline-perfused animals. One hemisphere was prepared for immunohistochemical analysis and the second was used for the quantitation of Aβ and APP levels. To measure the concentrations of various forms of beta amyloid peptide and amyloid precursor protein, the hemisphere was dissected and

homogenates of the hippocampal, cortical, and cerebellar
regions were prepared in 5 M guanidine. These were diluted
and the level of amyloid or APP was quantitated by comparison
to a series of dilutions of standards of Aβ peptide or APP of
5   known concentrations in an ELISA format.

The median concentration of total Aβ for the control group
immunized with PBS was 5.8-fold higher in the hippocampus than
in the cortex (median of 24,318 ng/g hippocampal tissue
compared to 4,221 ng/g for the cortex). The median level in
10   the cerebellum of the control group (23.4 ng/g tissue) was
about 1,000-fold lower than in the hippocampus. These levels
are similar to those that we have previously reported for
heterozygous PDAPP transgenic mice of this age (Johnson-Woods
et al., 1997, supra).

15   For the cortex, a subset of treatment groups had median
total Aβ and Aβ1-42 levels which differed significantly from
those of the control group (p < 0.05), those animals receiving
AN1792, rodent Aβ1-42 or the Aβ1-5 peptide conjugate as shown
in **Fig. 11**. The median levels of total Aβ were reduced by
20   75%, 79% and 61%, respectively, compared to the control for
these treatment groups. There were no discernable
correlations between Aβ-specific antibody titers and Aβ levels
in the cortical region of the brain for any of the groups.

In the hippocampus, the median reduction of total Aβ
25   associated with AN1792 treatment (46%, p = 0.0543) was not as
great as that observed in the cortex (75%, p = 0.0021).
However, the magnitude of the reduction was far greater in the
hippocampus than in the cortex, a net reduction of 11,186 ng/g
tissue in the hippocampus versus 3,171 ng/g tissue in the
30   cortex. For groups of animals receiving rodent Aβ1-42 or
Aβ1-5, the median total Aβ levels were reduced by 36% and 26%,
respectively. However, given the small group sizes and the
high variability of the amyloid peptide levels from animal to
animal within both groups, these reductions were not
35   significant. When the levels of Aβ1-42 were measured in the
hippocampus, none of the treatment-induced reductions reached
significance. Thus, due to the smaller Aβ burden in the
cortex, changes in this region are a more sensitive indicator

of treatment effects. The changes in Aβ levels measured by
ELISA in the cortex are similar, but not identical, to the
results from the immunohistochemical analysis (see below).

Total Aβ was also measured in the cerebellum, a region
5   typically unaffected in the AD pathology. None of the median
Aβ concentrations of any of the groups immunized with the
various Aβ peptides or the APP derivative differed from that
of the control group in this region of the brain. This result
suggests that non-pathological levels of Aβ are unaffected by
10   treatment.

APP concentration was also determined by ELISA in the
cortex and cerebellum from treated and control mice. Two
different APP assays were utilized. The first, designated
APP-α/FL, recognizes both APP-alpha (α, the secreted form of
15   APP which has been cleaved within the Aβ sequence), and full-
length forms (FL) of APP, while the second recognizes only
APP-α. In contrast to the treatment-associated diminution of
Aβ in a subset of treatment groups, the levels of APP were
unchanged in all of the treated compared to the control
20   animals. These results indicate that the immunizations with
Aβ peptides are not depleting APP; rather the treatment effect
is specific to Aβ.

In summary, total Aβ and Aβ1-42 levels were significantly
reduced in the cortex by treatment with AN1792, rodent Aβ1-42
25   or Aβ1-5 conjugate. In the hippocampus, total Aβ was
significantly reduced only by AN1792 treatment. No other
treatment-associated changes in Aβ or APP levels in the
hippocampal, cortical or cerebellar regions were significant.

    2. Histochemical Analyses
30   Brains from a subset of six groups were prepared for
immunohistochemical analysis, three groups immunized with the
Aβ peptide conjugates Aβ1-5, Aβ1-12, and Aβ13-28; two groups
immunized with the full length Aβ aggregates AN1792 and AN1528
and the PBS-treated control group. The results of image
35   analyses of the amyloid burden in brain sections from these
groups are shown in Fig. 12. There were significant
reductions of amyloid burden in the cortical regions of three

of the treatment groups versus control animals. The greatest
reduction of amyloid burden was observed in the group
receiving AN1792 where the mean value was reduced by 97% (p =
0.001). Significant reductions were also observed for those
animals treated with AN1528 (95%, p = 0.005) and the Aβ1-5
peptide conjugate (67%, p = 0.02).

The results obtained by quantitation of total Aβ or Aβ1-42
by ELISA and amyloid burden by image analysis differ to some
extent. Treatment with AN1528 had a significant impact on the
level of cortical amyloid burden when measured by quantitative
image analysis but not on the concentration of total Aβ in the
same region when measured by ELISA. The difference between
these two results is likely to be due to the specificities of
the assays. Image analysis measures only insoluble Aβ
aggregated into plaques. In contrast, the ELISA measures all
forms of Aβ, both soluble and insoluble, monomeric and
aggregated. Since the disease pathology is thought to be
associated with the insoluble plaque-associated form of Aβ,
the image analysis technique may have more sensitivity to
reveal treatment effects. However since the ELISA is a more
rapid and easier assay, it is very useful for screening
purposes. Moreover it may reveal that the treatment-
associated reduction of Aβ is greater for plaque-associated
than total Aβ.

To determine if the Aβ-specific antibodies elicited by
immunization in the treated animals reacted with deposited
brain amyloid, a subset of the sections from the treated
animals and the control mice were reacted with an antibody
specific for mouse IgG. In contrast to the PBS group, Aβ-
containing plaques were coated with endogenous IgG for animals
immunized with the Aβ peptide conjugates Aβ1-5, Aβ1-12, and
Aβ13-28; and the full length Aβ aggregates AN1792 and AN1528.
Brains from animals immunized with the other Aβ peptides or
the APP peptide pBx6 were not analyzed by this assay.

3.   <u>Measurement of Antibody Titers</u>

Mice were bled four to seven days following each
immunization starting after the second immunization, for a

total of five bleeds. Antibody titers were measured as Aβ1-
42-binding antibody using a sandwich ELISA with plastic multi-
well plates coated with Aβ1-42. As shown in Fig. 13, peak
antibody titers were elicited following the fourth dose for
those four vaccines which elicited the highest titers of
AN1792-specific antibodies: AN1792 (peak GMT: 94,647), AN1528
(peak GMT: 88,231), Aβ1-12 conjugate (peak GMT: 47,216)and
rodent Aβ1-42 (peak GMT: 10,766). Titers for these groups
declined somewhat following the fifth and sixth doses. For
the remaining five immunogens, peak titers were reached
following the fifth or the sixth dose and these were of much
lower magnitude than those of the four highest titer groups:
Aβ1-5 conjugate (peak GMT: 2,356), pBx6 (peak GMT: 1,986),
Aβ13-28 conjugate (peak GMT: 1,183), Aβ33-42 conjugate (peak
GMT: 658), Aβ25-35 (peak GMT: 125). Antibody titers were also
measured against the homologous peptides using the same ELISA
sandwich format for a subset of the immunogens, those groups
immunized with Aβ1-5, Aβ13-28, Aβ25-35, Aβ33-42 or rodent
Aβ1-42. These titers were about the same as those measured
against Aβ1-42 except for the rodent Aβ1-42 immunogen in which
case antibody titers against the homologous immunogen were
about two-fold higher. The magnitude of the AN1792-specific
antibody titer of individual animals or the mean values of
treatment groups did not correlate with efficacy measured as
the reduction of Aβ in the cortex.

4. Lymphoproliferative Responses

Aβ-dependent lymphoproliferation was measured using spleen
cells harvested approximately one week following the final,
sixth, immunization. Freshly harvested cells, 10^5 per well,
were cultured for 5 days in the presence of Aβ1-40 at a
concentration of 5 μM for stimulation. Cells from a subset of
seven of the ten groups were also cultured in the presence of
the reverse peptide, Aβ40-1. As a positive control,
additional cells were cultured with the T cell mitogen, PHA,
and, as a negative control, cells were cultured without added
peptide.

Lymphocytes from a majority of the animals proliferated in response to PHA. There were no significant responses to the Aβ40-1 reverse peptide. Cells from animals immunized with the larger aggregated Aβ peptides, AN1792, rodent Aβ1-42 and

5   AN1528 proliferated robustly when stimulated with Aβ1-40 with the highest cpm in the recipients of AN1792. One animal in each of the groups immunized with Aβ1-12 conjugate, Aβ13-28 conjugate and Aβ25-35 proliferated in response to Aβ1-40. The remaining groups receiving Aβ1-5 conjugate, Aβ33-42 conjugate

10   pBx6 or PBS had no animals with an Aβ-stimulated response. These results are summarized in Table 5 below.

Table 5			
Immunogen	Conjugate	Aβ Amino Acids	Responders
Aβ1-5	yes	5-mer	0/7
Aβ1-12	yes	12-mer	1/8
Aβ13-28	yes	16-mer	1/9
Aβ25-35		11-mer	1/9
Aβ33-42	yes	10-mer	0/10
Aβ1-40		42-mer	5/8
Aβ1-42		42-mer	9/9
r Aβ1-42		42-mer	8/8
pBx6			0/8
PBS		0-mer	0/8

These results show that AN1792 and AN1528 stimulate strong

25   T cell responses, most likely of the CD4$^+$ phenotype. The absence of an Aβ-specific T cell response in animals immunized with Aβ1-5 is not surprising since peptide epitopes recognized by CD4$^+$ T cells are usually about 15 amino acids in length, although shorter peptides can sometimes function with less

30   efficiency. Thus the majority of helper T cell epitopes for the four conjugate peptides are likely to reside in the IgG conjugate partner, not in the Aβ region. This hypothesis is supported by the very low incidence of proliferative responses for animals in each of these treatment groups. Since the Aβ1-

35   5 conjugate was effective at significantly reducing the level

of Aβ in the brain, in the apparent absence of Aβ-specific T
cells, the key effector immune response induced by
immunization with this peptide appears to be antibody.

Lack of T-cell and low antibody response from fusion peptide
pBx6, encompassing APP amino acids 592-695 including all of
the Aβ residues may be due to the poor immunogenicity of this
particular preparation. The poor immunogenicity of the Aβ25-
35 aggregate is likely due to the peptide being too small to
be likely to contain a good T cell epitope to help the
induction of an antibody response. If this peptide were
conjugated to a carrier protein, it would probably be more
immunogenic.

V. Preparation of Polyclonal Antibodies for Passive
    Protection

20 non-transgenic mice are immunized with Aβ or other
immunogen, optionally plus adjuvant, and are euthanized at 4-5
months. Blood is collected from immunized mice. Optionally,
IgG is separated from other blood components. Antibody
specific for the immunogen may be partially purified by
affinity chromatography. An average of about 0.5-1 mg of
immunogen-specific antibody is obtained per mouse, giving a
total of 5-10 mg.

VI. Passive Immunization with Antibodies to Aβ

Groups of 7-9 month old PDAPP mice each are injected with
0.5 mg in PBS of polyclonal anti-Aβ or specific anti-Aβ
monoclonals as shown below. All antibody preparations are
purified to have low endotoxin levels. Monoclonals can be
prepared against a fragment by injecting the fragment or
longer form of Aβ into a mouse, preparing hybridomas and
screening the hybridomas for an antibody that specifically

binds to a desired fragment of Aβ without binding to other
nonoverlapping fragments of Aβ.

Table 6

Antibody	Epitope
2H3	Aβ 1-12
10D5	Aβ 1-12
266	Aβ 13-28
21F12	Aβ 33-42
Mouse polyclonal anti-human Aβ42	Anti-Aggregated Aβ42

Mice are injected ip as needed over a 4 month period to
maintain a circulating antibody concentration measured by
ELISA titer of greater than 1/1000 defined by ELISA to Aβ42 or
other immunogen. Titers are monitored as above and mice are
euthanized at the end of 4 months of injections.
Histochemistry, Aβ levels and toxicology are performed post
mortem. Ten mice are used per group.

VII. Comparison of Different Adjuvants

This examples compares CFA, alum, an oil-in water emulsion
and MPL for capacity to stimulate an immune response.

A. <u>Materials and Methods</u>

1. <u>Study Design</u>

One hundred female Hartley strain six-week old guinea pigs, obtained from Elm Hill, were sorted into ten groups to be immunized with AN1792 or a palmitoylated derivative thereof combined with various adjuvants. Seven groups received injections of AN1792 (33μg unless otherwise specified) combined with a) PBS, b) Freund's adjuvant, c) MPL, d) squalene, e) MPL/squalene f) low dose alum, or g) high dose alum (300μg AN1792). Two groups received injections of a palmitoylated derivative of AN1792 (33 μg) combined with a) PBS or b) squalene. A final, tenth group received PBS alone without antigen or additional adjuvant. For the group receiving Freund's adjuvant, the first dose was emulsified with CFA and the remaining four doses with IFA. Antigen was administered at a dose of 33 μg for all groups except the high dose alum group, which received 300 μg of AN1792. Injections were administered intraperitoneally for CFA/IFA and intramuscularly in the hind limb quadriceps alternately on the right and left side for all other groups. The first three doses were given on a biweekly schedule followed by two doses at a monthly interval). Blood was drawn six to seven days following each immunization, starting after the second dose, for measurement of antibody titers.

2. <u>Preparation of Immunogens</u>

Two mg Aβ42 (California Peptide, Lot ME0339) was added to 0.9 ml of deionized water and the mixture was vortexed to generate a relatively uniform suspension. A 100 μl aliquot of 10X PBS (1X PBS, 0.15 M NaCl, 0.01 M sodium phosphate, pH 7.5) was added. The suspension was vortexed again and incubated overnight at 37°C for use the next day. Unused Aβ1-42 was stored with desiccant as a lyophilized powder at -20°C.

A palmitoylated derivative of AN1792 was prepared by
coupling palmitic anhydride, dissolved in dimethyl formamide,
to the amino terminal residue of AN1792 prior to removal of
the nascent peptide from the resin by treatment with
5   hydrofluoric acid.

To prepare vaccine doses with Complete Freund's adjuvant
(CFA) (group 2), 33 μg of AN1792 in 200 μl PBS was emulsified
1:1 (vol:vol) with CFA in a final volume of 400 μl for the
first immunization. For subsequent immunizations, the antigen
10   was similarly emulsified with Incomplete Freund's adjuvant
(IFA).

To prepare vaccine doses with MPL for groups 5 and 8,
lyophilized powder (Ribi ImmunoChem Research, Inc., Hamilton,
MT) was added to 0.2% aqueous triethylamine to a final
15   concentration of 1 mg/ml and vortexed. The mixture was heated
to 65 to 70°C for 30 sec to create a slightly opaque uniform
suspension of micelles. The solution was freshly prepared for
each set of injections. For each injection in group 5, 33 μg
of AN1792 in 16.5 μl PBS, 50 μg of MPL (50 μl) and 162 μl of
20   PBS were mixed in a borosilicate tube immediately before use.

To prepare vaccine doses with the low oil-in-water
emulsion, AN1792 in PBS was added to 5% squalene, 0.5% Tween
80., 0.5% Span 85 in PBS to reach a final single dose
concentration of 33 μg AN1792 in 250 μl (group 6). The mixture
25   was emulsified by passing through a two-chambered hand-held
device 15 to 20 times until the emulsion droplets appeared to
be about equal in diameter to a 1.0 μm diameter standard latex
bead when viewed under a microscope. The resulting suspension
was opalescent, milky white. The emulsions were freshly
30   prepared for each series of injections. For group 8, MPL in
0.2% triethylamine was added at a concentration of 50 μg per
dose to the squalene and detergent mixture for emulsification
as noted above. For the palmitoyl derivative (group 7), 33 μg
per dose of palmitoyl-NH-Aβ1-42 was added to squalene and
35   vortexed. Tween 80 and Span 85 were then added with

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vortexing. This mixture was added to PBS to reach final
concentrations of 5% squalene, 0.5% Tween 80, 0.5% Span 85 and
the mixture was emulsified as noted above.

5   To prepare vaccine doses with alum (groups 9 and 10),
AN1792 in PBS was added to Alhydrogel (aluminum hydroxide gel,
Accurate, Westbury, NY) to reach concentrations of 33 μg (low
dose, group 9) or 300 μg (high dose, group 10) AN1792 per 5 mg
of alum in a final dose volume of 250 μl. The suspension was
gently mixed for 4 hr at RT.

10   3. Measurement of Antibody Titers

Guinea pigs were bled six to seven days following
immunization starting after the second immunization for a
total of four bleeds. Antibody titers against Aβ42 were
measured by ELISA as described in General Materials and
15   Methods.

4. Tissue Preparation

. After about 14 weeks, all guinea pigs were administered
CO_2. Cerebrospinal fluid was collected and the brains were
20   removed and three brain regions (hippocampus, cortex and
cerebellum) were dissected and used to measure the
concentration of total Aβ protein using ELISA.

B. <u>Results</u>

  1. <u>Antibody Responses</u>

      There was a wide range in the potency of the various
adjuvants when measured as the antibody response to AN1792
5   following immunization. As shown in **Fig. 14**, when AN1792 was
administered in PBS, no antibody was detected following two or
three immunizations and negligible responses were detected
following the fourth and fifth doses with geometric mean
titers (GMTs) of only about 45. The o/w emulsion induced
10 modest titers following the third dose (GMT 255) that were
maintained following the fourth dose (GMT 301) and fell with
the final dose (GMT 54). There was a clear antigen dose
response for AN1792 bound to alum with 300 μg being more
immunogenic at all time points than 33 μg. At the peak of the
15 antibody response, following the fourth immunization, the
difference between the two doses was 43% with GMTs of about
1940 (33 μg) and 3400 (300 μg). The antibody response to 33
μg AN1792 plus MPL was very similar to that generated with
almost a ten-fold higher dose of antigen (300 μg) bound to
20 alum. The addition of MPL to an o/w emulsion decreased the
potency of the vaccine relative to that with MPL as the sole
adjuvant by as much as 75%. A palmitoylated derivative of
AN1792 was completely non-immunogenic when administered in PBS
and gave modest titers when presented in an o/w emulsion with
25 GMTs of 340 and 105 for the third and fourth bleeds. The
highest antibody titers were generated with Freund's adjuvant
with a peak GMT of about 87,000, a value almost 30-fold
greater than the GMTs of the next two most potent vaccines,
MPL and high dose AN1792/alum.

30   The most promising adjuvants identified in this study are
MPL and alum. Of these two, MPL appears preferable because a
10-fold lower antigen dose was required to generate the same
antibody response as obtained with alum. The response can be
increased by increasing the dose of antigen and /or adjuvant
35 and by optimizing the immunization schedule. The o/w emulsion

was a very weak adjuvant for AN1792 and adding an o/w emulsion to MPL adjuvant diminished the intrinsic adjuvant activity of MPL alone.

2. Aβ Levels In The Brain

5   At about 14 weeks the guinea pigs were deeply anesthetized, the cerebrospinal fluid (CSF) was drawn and brains were excised from animals in a subset of the groups, those immunized with Freund's adjuvant (group 2), MPL (group 5), alum with a high dose, 300 μg, of AN1792 (group 10) and

10   the PBS immunized control group (group 3). To measure the level of Aβ peptide, one hemisphere was dissected and homogenates of the hippocampal, cortical, and cerebellar regions were prepared in 5 M guanidine. These were diluted and quantitated by comparison to a series of dilutions of Aβ

15   standard protein of known concentrations in an ELISA format. The levels of Aβ protein in the hippocampus, the cortex and the cerebellum were very similar for all four groups despite the wide range of antibody responses to Aβ elicited by these vaccines. Mean Aβ levels of about 25 ng/g tissue were

20   measured in the hippocampus, 21 ng/g in the cortex, and 12 ng/g in the cerebellum. Thus, the presence of a high circulating antibody titer to Aβ for almost three months in some of these animals did not alter the total Aβ levels in their brains. The levels of Aβ in the CSF were also quite

25   similar between the groups. The lack of large effect of AN1792 immunization on endogenous Aβ indicates that the immune response is focused on pathological formations of Aβ.

VIII. Immune Response to Different Adjuvants in Mice

        Six-week old female Swiss Webster mice were used for this
30   study with 10-13 animals per group. Immunizations were given

on days 0, 14, 28, 60, 90 and 20 administered subcutaneously in a dose volume of 200 μl. PBS was used as the buffer for all formulations. Animals were bleed seven days following each immunization starting after the second dose for analysis

5 of antibody titers by ELISA. The treatment regime of each group is summarized in Table 7.

Table 7

Experimental Design of Betabloc Study 010					
Group	N [a]	Adjuvant [b]	Dose	Antigen	Dose (µg)
1	10	MPL	12.5 µg	AN1792	33
2	10	MPL	25 µg	AN1792	33
3	10	MPL	50 µg	AN1792	33
4	13	MPL	125 µg	AN1792	33
5	13	MPL	50 µg	AN1792	150
6	13	MPL	50 µg	AN1528	33
7	10	PBS		AN1792	33
8	10	PBS		none	
9	10	Squalene emulsified	5%	AN1792	33
10	10	Squalene admixed	5%	AN1792	33
11	10	Alum	2 mg	AN1792	33
12	13	MPL + Alum	50 µg/2 mg	AN1792	33
13	10	QS21	5 µg	AN1792	33
14	10	QS21	10 µg	AN1792	33
15	10	QS21	25 µg	AN1792	33
16	13	QS21	25 µg	AN1792	150
17	13	QS21	25 µg	AN1528	33
18	13	QS21 + MPL	25 µg/50 µg	AN1792	33
19	13	QS21 + Alum	25 µg/2 mg	AN1792	33

Footnotes:

[a] Number of mice in each group at the initiation of the experiment.

[b] The adjuvants are noted. The buffer for all these formulations was PBS. For group 8, there was no adjuvant and no antigen.

The ELISA titers of antibodies against Aβ42 in each group are shown in Table 8 below.

Table 8.

Geometric Mean Antibody Titers					
Week of Bleed					
Treatment Group	2.9	5.0	8.7	12.9	16.7
1	248	1797	2577	6180	4177
2	598	3114	3984	5287	6878
3	1372	5000	7159	12333	12781
4	1278	20791	14368	20097	25631
5	3288	26242	13229	9315	23742
6	61	2536	2301	1442	4504
7	37	395	484	972	2149
8	25	25	25	25	25
9	25	183	744	952	1823
10	25	89	311	513	817
11	29	708	2618	2165	3666
12	198	1458	1079	612	797
13	38	433	566	1080	626
14	104	541	3247	1609	838
15	212	2630	2472	1224	1496
16	183	2616	6680	2085	1631
17	28	201	375	222	1540
18	31699	15544	23095	6412	9059
19	63	243	554	299	441

The table shows that the highest titers were obtained for groups 4, 5 and 18, in which the adjuvants were 125 μg MPL, 50 μg MPL and QS21 plus MPL.

IX. Therapeutic Efficacy of Different Adjuvants

A therapeutic efficacy study was conducted in PDAPP transgenic mice with a set of adjuvants suitable for use in humans to determine their ability to potentiate immune responses to Aβ and to induce the immune-mediated clearance of amyloid deposits in the brain.

One hundred eighty male and female, 7.5- to 8.5-month old heterozygous PDAPP transgenic mice were obtained from Charles River Laboratories. The mice were sorted into nine groups containing 15 to 23 animals per group to be immunized with

5   AN1792 or AN1528 combined with various adjuvants. Animals were distributed to match the gender, age, and parentage of the animals within the groups as closely as possible. The adjuvants included alum, MPL, and QS21, each combined with both antigens, and Freund's adjuvant (FA) combined with only

10   AN1792. An additional group was immunized with AN1792 formulated in PBS buffer plus the preservative thimerosal without adjuvant. A ninth group was immunized with PBS alone as a negative control.

Preparation of aggregated Aβ peptides: human Aβ1-40

15   (AN1528; California Peptides Inc., Napa, CA; Lot ME0541) and human Aβ1-42 (AN1792; California Peptides Inc., Lot ME0439) peptides were freshly solubilized for the preparation of each set of injections from lyophilized powders that had been stored desiccated at -20°C. For this purpose, two mg of

20   peptide were added to 0.9 ml of deionized water and the mixture was vortexed to generate a relatively uniform solution or suspension. AN1528 was soluble at this step, in contrast to AN1792. A 100 μl aliquot of 10X PBS (1X PBS: 0.15 M NaCl, 0.01 M sodium phosphate, pH 7.5) was then added at which point

25   AN1528 began to precipitate. The suspensions were vortexed again and incubated overnight at 37°C for use the next day.

To prepare vaccine doses with alum (Groups 1 and 5), Aβ peptide in PBS was added to Alhydrogel (two percent aqueous aluminum hydroxide gel, Sargeant, Inc., Clifton, NJ) to reach

30   concentrations of 100 μg Aβ peptide per 1 mg of alum. 10X PBS was added to a final dose volume of 200 μl in 1X PBS. The suspension was then gently mixed for approximately 4 hr at RT prior to injection.

To prepare vaccine doses for with MPL (Groups 2 and 6),

35   lyophilized powder (Ribi ImmunoChem Research, Inc., Hamilton,

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MT; Lot 67039-E0896B) was added to 0.2% aqueous triethylamine
to a final concentration of 1 mg/ml and vortexed. The mixture
was heated to 65 to 70°C for 30 sec to create a slightly
opaque uniform suspension of micelles. The solution was
5   stored at 4°C. For each set of injections, 100 μg of peptide
per dose in 50 μl PBS, 50 μg of MPL per dose (50 μl) and 100
μl of PBS per dose were mixed in a borosilicate tube
immediately before use.

        To prepare vaccine doses with QS21 (Groups 3 and 7),
10   lyophilized powder (Aquila, Framingham, MA; Lot A7018R) was
added to PBS, pH 6.6-6.7 to a final concentration of 1 mg/ml
and vortexed. The solution was stored at -20°C. For each set
of injections, 100 μg of peptide per dose in 50 μl PBS, 25 μg
of QS21 per dose in 25 μl PBS and 125 μl of PBS per dose were
15   mixed in a borosilicate tube immediately before use.

        To prepare vaccine doses with Freund's Adjuvant (Group 4),
100 μg of AN1792 in 200 μl PBS was emulsified 1:1 (vol:vol)
with Complete Freund's Adjuvant (CFA) in a final volume of 400
μl for the first immunization. For subsequent immunizations,
20   the antigen was similarly emulsified with Incomplete Freund's
Adjuvant (IFA). For the vaccines containing the adjuvants
alum, MPL or QS21, 100 μg per dose of AN1792 or AN1528 was
combined with alum (1 mg per dose) or MPL (50 μg per dose) or
QS21 (25 μg per dose) in a final volume of 200 μl PBS and
25   delivered by subcutaneous inoculation on the back between the
shoulder blades. For the group receiving FA, 100 μg of AN1792
was emulsified 1:1 (vol:vol) with Complete Freund's adjuvant
(CFA) in a final volume of 400 μl and delivered
intraperitoneally for the first immunization, followed by a
30   boost of the same amount of immunogen in Incomplete Freund's
adjuvant (IFA) for the subsequent five doses. For the group
receiving AN1792 without adjuvant, 10 μg AN1792 was combined
with 5 μg thimerosal in a final volume of 50 μl PBS and
delivered subcutaneously. The ninth, control group received
35   only 200 μl PBS delivered subcutaneously. Immunizations were
given on a biweekly schedule for the first three doses, then

on a monthly schedule thereafter on days 0, 16, 28, 56, 85 and
112.   Animals were bled six to seven days following each
immunization starting after the second dose for the
measurement of antibody titers.   Animals were euthanized

5   approximately one week after the final dose.   Outcomes were
measured by ELISA assay of Aβ and APP levels in brain and by
immunohistochemical evaluation of the presence of amyloid
plaques in brain sections.   In addition, Aβ-specific antibody
titers, and Aβ-dependent proliferative and cytokine responses

10   were determined.

. Table 9 shows that the highest antibody titers to Aβ1-42
were elicited with FA and AN1792, titers which peaked ·
following the fourth immunization (peak GMT: 75,386) and then
declined by 59% after the final, sixth immunization.   The peak

15   mean titer elicited by MPL with AN1792 was 62% lower than that
generated with FA (peak GMT: 28,867) and was also reached
early in the immunization scheme, after 3 doses, followed by a
decline to 28% of the peak value after the sixth immunization.
The peak mean titer generated with QS21 combined with AN1792

20   (GMT: 1,511) was about 5-fold lower than obtained with MPL.
In addition, the kinetics of the response were slower, since
an additional immunization was required to reach the peak
response.   Titers generated by alum-bound AN1792 were
marginally greater than those obtained with QS21 and the .

25   response kinetics were more rapid.   For AN1792 delivered in
PBS with thimerosal the frequency and size of titers were
barely greater than that for PBS alone.   The peak titers
generated with MPL and AN1528 (peak GMT 3099) were about 9-
fold lower than those with AN1792.   Alum-bound AN1528 was very

30   poorly immunogenic with low titers generated in only some of
the animals.   No antibody responses were observed in the
control animals immunized with PBS alone.

Table 9

Geometric Mean Antibody Titers[a]				
Week of Bleed				
Treatment	3.3	5.0	9.0	13.0	17.0
Alum/ AN1792	102 (12/21)[b]	1,081 (17/20)	2,366 (21/21)	1,083 (19/21)	572 (18/21)
MPL/ AN1792	6241 (21/21)	28,867 (21/21)	1,1242 (21/21)	5,665 (20/20)	8,204 (20/20)
QS21/ AN1792	30 (1/20)	227 (10/19)	327 (10/19)	1,511 (17/18)	1,188 (14/18)
CFA/ AN1792	10,076 (15/15)	61,279 (15/15)	75,386 (15/15)	41,628 (15/15)	30,574 (15/15)
Alum/ AN1528	25 (0/21)	33 (1/21)	39 (3/20)	37 (1/20)	31 (2/20)
MPL/ AN1528	184 (15/21)	2,591 (20/21)	1,653 (21/21)	1,156 (20/20)	3,099 (20/20)
QS21/ AN1528	29 (1/22)	221 (13/22)	51 (4/22)	820 (20/22)	2,994 (21/22)
PBS plus Thimerosal	25 (0/16)	33 (2/16)	39 (4/16)	37 (3/16)	47 (4/16)
PBS	25 (0/16)	25 (0/16)	25 (0/15)	25 (0/12)	25 (0/16)

Footnotes:

[a] Geometric mean antibody titers measured against Aβ1-42

[b] Number of responders per group

The results of AN1792 or AN1592 treatment with various adjuvants, or thimerosal on cortical amyloid burden in 12-month old mice determined by ELISA are shown in Fig. 15.   In PBS control PDAPP mice, the median level of total Aβ in the

cortex at 12 months was 1,817 ng/g. Notably reduced levels of
Aβ were observed in mice treated with AN1792 plus CFA/IFA,
AN1792 plus alum, AN1792 plus MPL and QS21 plus AN1792. The
reduction reached statistical significance (p<0.05) only for

5   AN1792 plus CFA/IFA. However, as shown in Examples I and III,
the effects of immunization in reducing Aβ levels become
substantially greater in 15 month and 18 month old mice.
Thus, it is expected that at least the AN1792 plus alum,
AN1792 plus MPL and AN1792 plus QS21 compositions will achieve

10   statistical significance in treatment of older mice. By
contrast, the AN1792 plus the preservative thimerosal showed a
median level of Aβ about the same as that in the PBS treated
mice. Similar results were obtained when cortical levels of
Aβ42 were compared. The median level of Aβ42 in PBS controls

15   was 1624 ng/g. Notably reduced median levels of 403, 1149,
620 and 714 were observed in the mice treated with AN1792 plus
CFA/IFA, AN1792 plus alum, AN1792 plus MPL and AN1792 plus
QS21 respectively, with the reduction achieving statistical
significance (p=0.05) for the AN1792 CFA/IFA treatment group.

20   The median level in the AN1792 thimerosal treated mice was
1619 ng/g Aβ42.

     X.   Toxicity Analysis

     Tissues were collected for histopathologic examination at
the termination of studies described in Examples 2, 3 and 7.

25   In addition, hematology and clinical chemistry were performed
on terminal blood samples from Examples 3 and 7. Most of the
major organs were evaluated, including brain, pulmonary,
lymphoid, gastrointestinal, liver, kidney, adrenal and gonads.
Although sporadic lesions were observed in the study animals,

30   there were no obvious differences, either in tissues affected
or lesion severity, between AN1792 treated and untreated
animals. There were no unique histopathological lesions noted
in AN-1782-immunized animals compared to PBS-treated or
untreated animals. There were also no differences in the

clinical chemistry profile between adjuvant groups and the PBS treated animals in Example 7. Although there were significant increases in several of the hematology parameters between animals treated with AN1792 and Freund's adjuvant in Example 7

5   relative to PBS treated animals, these type of effects are expected from Freund's adjuvant treatment and the accompanying peritonitis and do not indicate any adverse effects from AN1792 treatment. Although not part of the toxicological evaluation, PDAPP mouse brain pathology was extensively

10   examined as part of the efficacy endpoints. No sign of treatment related adverse effect on brain morphology was noted in any of the studies. These results indicate that AN1792 treatment is well tolerated and at least substantially free of side effects.

15   XI. <u>Prevention and Treatment of Subjects</u>

A single-dose phase I trial is performed to determine safety. A therapeutic agent is administered in increasing dosages to different patients starting from about 0.01 the level of presumed efficacy, and increasing by a factor of

20   three until a level of about 10 times the effective mouse dosage is reached.

A phase II trial is performed to determine therapeutic efficacy. Patients with early to mid Alzheimer's Disease defined using Alzheimer's disease and Related Disorders

25   Association (ADRDA) criteria for probable AD are selected. Suitable patients score in the 12-26 range on the Mini-Mental State Exam (MMSE). Other selection criteria are that patients are likely to survive the duration of the study and lack complicating issues such as use of concomitant medications

30   that may interfere. Baseline evaluations of patient function are made using classic psychometric measures, such as the MMSE, and the ADAS, which is a comprehensive scale for evaluating patients with Alzheimer's Disease status and

function.   These psychometric scales provide a measure of
progression of the Alzheimer's condition.   Suitable
qualitative life scales can also be used to monitor treatment.
Disease progression can also be monitored by MRI.   Blood
5   profiles of patients can also be monitored including assays of
immunogen-specific antibodies and T-cells responses.

Following baseline measures, patients begin receiving
treatment.   They are randomized and treated with either
therapeutic agent or placebo in a blinded fashion.   Patients
10   are monitored at least every six months.   Efficacy is
determined by a significant reduction in progression of a
treatment group relative to a placebo group.

A second phase II trial is performed to evaluate conversion
of patients from non-Alzheimer's Disease early memory loss,
15   sometimes referred to as age-associated memory impairment
(AAMI), to probable Alzheimer's disease as defined as by ADRDA
criteria.   Patients with high risk for conversion to
Alzheimer's Disease are selected from a non-clinical
population by screening reference populations for early signs
20   of memory loss or other difficulties associated with pre-
Alzheimer's symptomatology, a family history of Alzheimer's
Disease, genetic risk factors, age, sex, and other features
found to predict high-risk for Alzheimer's Disease.   Baseline
scores on suitable metrics including the MMSE and the ADAS
25   together with other metrics designed to evaluate a more normal
population are collected.   These patient populations are
divided into suitable groups with placebo comparison against
dosing alternatives with the agent.   These patient populations
are followed at intervals of about six months, and the
30   endpoint for each patient is whether or not he or she converts
to probable Alzheimer's Disease as defined by ADRDA criteria
at the end of the observation.

XII.   General Materials and Methods

1.   Measurement of Antibody Titers

Mice were bled by making a small nick in the tail vein and
collecting about 200 μl of blood into a microfuge tube.
Guinea pigs were bled by first shaving the back hock area and
then using an 18 gauge needle to nick the metatarsal vein and
collecting the blood into microfuge tubes. Blood was allowed
to clot for one hr at room temperature (RT), vortexed, then
centrifuged at 14,000 x g for 10 min to separate the clot from
the serum. Serum was then transferred to a clean microfuge
tube and stored at 4° C until titered.

Antibody titers were measured by ELISA. 96-well microtiter
plates (Costar EIA plates) were coated with 100 μl of a
solution containing either 10 μg/ml either Aβ42 or SAPP or
other antigens as noted in each of the individual reports in
Well Coating Buffer (0.1 M sodium phosphate, pH 8.5, 0.1%
sodium azide) and held overnight at RT. The wells were
aspirated and sera were added to the wells starting at a 1/100
dilution in Specimen Diluent (0.014 M sodium phosphate, pH
7.4, 0.15 M NaCl, 0.6% bovine serum albumin, 0.05%
thimerosal). Seven serial dilutions of the samples were made
directly in the plates in three-fold steps to reach a final
dilution of 1/218,700. The dilutions were incubated in the
coated-plate wells for one hr at RT. The plates were then
washed four times with PBS containing 0.05% Tween 20. The
second antibody, a goat anti-mouse Ig conjugated to
horseradish peroxidase (obtained from Boehringer Mannheim),
was added to the wells as 100 μl of a 1/3000 dilution in
Specimen Diluent and incubated for one hr at RT. Plates were
again washed four times in PBS, Tween 20. To develop the
chromogen, 100 μl of Slow TMB (3,3',5,5'-tetramethyl benzidine
obtained from Pierce Chemicals) was added to each well and
incubated for 15 min at RT. The reaction was stopped by the
addition of 25 μl of 2 M H_2SO_4. The color intensity was then
read on a Molecular Devices Vmax at (450 nm - 650 nm).

Titers were defined as the reciprocal of the dilution of
serum giving one half the maximum OD. Maximal OD was
generally taken from an initial 1/100 dilution, except in
cases with very high titers, in which case a higher initial
5   dilution was necessary to establish the maximal OD. If the
50% point fell between two dilutions, a linear extrapolation
was made to calculate the final titer. To calculate geometric
mean antibody titers, titers less than 100 were arbitrarily
assigned a titer value of 25.

10   2. Lymphocyte proliferation assay

Mice were anesthetized with isoflurane. Spleens were
removed and rinsed twice with 5 ml PBS containing 10% heat-
inactivated fetal bovine serum (PBS-FBS) and then homogenized
in a 50 μ Centricon unit (Dako A/S, Denmark) in 1.5 ml PBS-FBS
15   for 10 sec at 100 rpm in a Medimachine (Dako) followed by
filtration through a 100 μ pore size nylon mesh. Splenocytes
were washed once with 15 ml PBS-FBS, then pelleted by
centrifugation at 200 x g for 5 min. Red blood cells were
lysed by resuspending the pellet in 5 mL buffer containing
20   0.15 M NH_4Cl, 1 M $KHCO_3$, 0.1 M NaEDTA, pH 7.4 for five min at
RT. Leukocytes were then washed as above. Freshly isolated
spleen cells (10^5 cells per well) were cultured in triplicate
sets in 96-well U-bottomed tissue culture-treated microtiter
plates (Corning, Cambridge, MA) in RPMI 1640 medium (JRH
25   Biosciences, Lenexa, KS) supplemented with 2.05 mM L
glutamine, 1% Penicillin/Streptomycin, and 10% heat-
inactivated FBS, for 96 hr at 37°C. Various Aβ peptides, Aβ1-
16, Aβ1-40, Aβ1-42 or Aβ40-1 reverse sequence protein were
also added at doses ranging from 5 μM to 0.18 μM in four
30   steps. Cells in control wells were cultured with Concanavalin
A (Con A) (Sigma, cat. # C-5275, at 1 μg/ml) without added
protein. Cells were pulsed for the final 24 hr with ^{3}H-
thymidine (1 μCi/well obtained from Amersham Corp., Arlington
Heights IL). Cells were then harvested onto UniFilter plates

and counted in a Top Count Microplate Scintillation Counter
(Packard Instruments, Downers Grove, IL). Results are
expressed as counts per minute (cpm) of radioactivity
incorporated into insoluble macromolecules.

5   4.   Brain Tissue Preparation

         After euthanasia, the brains were removed and one
    hemisphere was prepared for immunohistochemical analysis,
    while three brain regions (hippocampus, cortex and cerebellum)
    were dissected from the other hemisphere and used to measure
10   the concentration of various Aβ proteins and APP forms using
    specific ELISAs (Johnson-Wood et al., supra).

         Tissues destined for ELISAs were homogenized in 10 volumes
    of ice-cold guanidine buffer (5.0 M guanidine-HCl, 50 mM Tris-
    HCl, pH 8.0). The homogenates were mixed by gentle agitation
15   using an Adams Nutator (Fisher) for three to four hr at RT,
    then stored at -20°C prior to quantitation of Aβ and APP.
    Previous experiments had shown that the analytes were stable
    under this storage condition, and that synthetic Aβ protein
    (Bachem) could be quantitatively recovered when spiked into
20   homogenates of control brain tissue from mouse littermates
    (Johnson-Wood et al., supra).

         5.   Measurement of Aβ Levels

         The brain homogenates were diluted 1:10 with ice cold
    Casein Diluent (0.25% casein, PBS, 0.05% sodium azide, 20
25   μg/ml aprotinin, 5 mM EDTA pH 8.0, 10 μg/ml leupeptin) and
    then centrifuged at 16,000 x g for 20 min at 4° C. The
    synthetic Aβ protein standards (1-42 amino acids) and the APP
    standards were prepared to include 0.5 M guanidine and 0.1%
    bovine serum albumin (BSA) in the final composition. The

"total" Aβ sandwich ELISA utilizes monoclonal antibody (mAβ) 266, specific for amino acids 13-28 of Aβ (Seubert, et al.), as the capture antibody, and biotinylated mAβ 3D6, specific for amino acids 1-5 of Aβ (Johnson-Wood, et al), as the

5   reporter antibody. The 3D6 mAβ does not recognize secreted APP or full-length APP, but detects only Aβ species with an amino-terminal aspartic acid. This assay has a lower limit of sensitivity of ˜50 ρg/ml (11 ρM) and shows no cross-reactivity to the endogenous murine Aβ protein at concentrations up to 1

10   ng/ml (Johnson-Wood et al., supra).

         The Aβ1-42 specific sandwich ELISA employs mAβ 21F12, specific for amino acids 33-42 of Aβ (Johnson-Wood, et al.), as the capture antibody. Biotinylated mAβ 3D6 is also the reporter antibody in this assay which has a lower limit of

15   sensitivity of about 125 ρg/ml (28 ρM, Johnson-Wood et al.). For the Aβ ELISAs, 100 μl of either mAβ 266 (at 10 μg/ml) or mAβ 21F12 at (5 μg/ml) was coated into the wells of 96-well immunoassay plates (Costar) by overnight incubation at RT. The solution was removed by aspiration and the wells were

20   blocked by the addition of 200 μl of 0.25% human serum albumin in PBS buffer for at least 1 hr at RT. Blocking solution was removed and the plates were stored desiccated at 4°C until used. The plates were rehydrated with Wash Buffer [Tris-buffered saline (0.15 M NaCl, 0.01 M Tris-HCl, pH 7.5), plus

25   0.05% Tween 20] prior to use. The samples and standards were added in triplicate aliquots of 100 μl per well and then incubated overnight at 4° C. The plates were washed at least three times with Wash Buffer between each step of the assay. The biotinylated mAβ 3D6, diluted to 0.5 μg/ml in Casein Assay

30   Buffer (0.25% casein, PBS, 0.05% Tween 20, pH 7.4), was added and incubated in the wells for 1 hr at RT. An avidin-horseradish peroxidase conjugate, (Avidin-HRP obtained from Vector, Burlingame, CA), diluted 1:4000 in Casein Assay Buffer, was added to the wells for 1 hr at RT. The

35   colorimetric substrate, Slow TMB-ELISA (Pierce), was added and allowed to react for 15 minutes at RT, after which the enzymatic reaction was stopped by the addition of 25 μl 2 N

H_2SO_4. The reaction product was quantified using a Molecular Devices Vmax measuring the difference in absorbance at 450 nm and 650 nm.

6.   Measurement of APP Levels

5   Two different APP assays were utilized. The first, designated APP-α/FL, recognizes both APP-alpha (α) and full-length (FL) forms of APP. The second assay is specific for APP-α. The APP-α/FL assay recognizes secreted APP including the first 12 amino acids of Aβ. Since the reporter antibody

10   (2H3) is not specific to the α-clip-site, occurring between amino acids 612-613 of APP695 (Esch et al., *Science* 248, 1122-1124 (1990)); this assay also recognizes full length APP (APP-FL). Preliminary experiments using immobilized APP antibodies to the cytoplasmic tail of APP-FL to deplete brain homogenates

15   of APP-FL suggest that approximately 30-40% of the APP-α/FL APP is FL (data not shown). The capture antibody for both the APP-α/FL and APP-α assays is mAβ 8E5, raised against amino acids 444 to 592 of the APP695 form (Games et al., *supra*). The reporter mAβ for the APP-α/FL assay is mAβ 2H3, specific

20   for amino acids 597-608 of APP695 (Johnson-Wood et al., *supra*) and the reporter antibody for the APP-α assay is a biotinylated derivative of mAβ 16H9, raised to amino acids 605 to 611 of APP. The lower limit of sensitivity of the APP-α/FL assay is about 11 ng/ml (150 ρM) (Johnson-Wood et al.) and

25   that of the APP-α specific assay is 22 ng/ml (0.3 nM). For both APP assays, mAβ 8E5 was coated onto the wells of 96-well EIA plates as described above for mAβ 266. Purified, recombinant secreted APP-α was used as the reference standard for the APP-α assay and the APP-α/FL assay (Esch et al.,

30   *supra*). The brain homogenate samples in 5 M guanidine were diluted 1:10 in ELISA Specimen Diluent (0.014 M phosphate buffer, pH 7.4, 0.6% bovine serum albumin, 0.05% thimerosal, 0.5 M NaCl, 0.1% NP40). They were then diluted 1:4 in Specimen Diluent containing 0.5 M guanidine. Diluted

homogenates were then centrifuged at 16,000 x g for 15 seconds
at RT. The APP standards and samples were added to the plate
in duplicate aliquots and incubated for 1.5 hr at RT. The
biotinylated reporter antibody 2H3 or 16H9 was incubated with
5   samples for 1 hr at RT. Streptavidin-alkaline phosphatase
(Boehringer Mannheim), diluted 1:1000 in specimen diluent, was
incubated in the wells for 1 hr at RT. The fluorescent
substrate 4-methyl-umbellipheryl-phosphate was added for a 30-
min RT incubation and the plates were read on a Cytofluor tm
10   2350 fluorimeter (Millipore) at 365 nm excitation and 450 nm
emission.

7.   Immunohistochemistry

Brains were fixed for three days at 4°C in 4%
paraformaldehyde in PBS and then stored from one to seven days
15   at 4°C in 1% paraformaldehyde, PBS until sectioned. Forty-
micron-thick coronal sections were cut on a vibratome at RT
and stored in cryoprotectant (30% glycerol, 30% ethylene
glycol in phosphate buffer) at -20°C prior to
immunohistochemical processing. For each brain, six sections
20   at the level of the dorsal hippocampus, each separated by
consecutive 240 μm intervals, were incubated overnight with
one of the following antibodies: (1) a biotinylated anti-Aβ
(mAβ, 3D6, specific for human Aβ) diluted to a concentration
of 2 μg/ml in PBS and 1% horse serum; or (2) a biotinylated
25   mAβ specific for human APP, 8E5, diluted to a concentration of
3 μg/ml in PBS and 1.0% horse serum; or (3) a mAβ specific for
glial fibrillary acidic protein (GFAP; Sigma Chemical Co.)
diluted 1:500 with 0.25% Triton X-100 and 1% horse serum, in
Tris-buffered saline, pH 7.4 (TBS); or (4) a mAβ specific for
30   CD11b, MAC-1 antigen, (Chemicon International) diluted 1:100
with 0.25% Triton X-100 and 1% rabbit serum in TBS; or (5) a
mAβ specific for MHC II antigen, (Pharmingen) diluted 1:100
with 0.25% Triton X-100 and 1% rabbit serum in TBS; or (6) a
rat mAβ specific for CD 43 (Pharmingen) diluted 1:100 with 1%

rabbit serum in PBS or (7) a rat mAβ specific for CD 45RA
(Pharmingen) diluted 1:100 with 1% rabbit serum in PBS; or (8)
a rat monoclonal Aβ specific for CD 45RB (Pharmingen) diluted
1:100 with 1% rabbit serum in PBS; or (9) a rat monoclonal Aβ

5   specific for CD 45 (Pharmingen) diluted 1:100 with 1% rabbit
serum in PBS; or (10) a biotinylated polyclonal hamster Aβ
specific for CD3e (Pharmingen) diluted 1:100 with 1% rabbit
serum in PBS or (11) a rat mAβ specific for CD3 (Serotec)
diluted 1:200 with 1% rabbit serum in PBS; or with (12) a

10   solution of PBS lacking a primary antibody containing 1%
normal horse serum.

Sections reacted with antibody solutions listed in 1,2 and
6-12 above were pretreated with 1.0% Triton X-100, 0.4%
hydrogen peroxide in PBS for 20 min at RT to block endogenous

15   peroxidase. They were next incubated overnight at 4°C with
primary antibody. Sections reacted with 3D6 or 8E5 or CD3e
mAβs were then reacted for one hr at RT with a horseradish
peroxidase-avidin-biotin-complex with kit components "A" and
"B" diluted 1:75 in PBS (Vector Elite Standard Kit, Vector

20   Labs, Burlingame, CA.). Sections reacted with antibodies
specific for CD 45RA, CD 45RB, CD 45, CD3 and the PBS solution
devoid of primary antibody were incubated for 1 hour at RT
with biotinylated anti-rat IgG (Vector) diluted 1:75 in PBS or
biotinylated anti-mouse IgG (Vector) diluted 1:75 in PBS,

25   respectively. Sections were then reacted for one hr at RT
with a horseradish peroxidase-avidin-biotin-complex with kit
components "A" and "B" diluted 1:75 in PBS (Vector Elite
Standard Kit, Vector Labs, Burlingame, CA.).

Sections were developed in 0.01% hydrogen peroxide, 0.05%

30   3,3'-diaminobenzidine (DAB) at RT. Sections destined for
incubation with the GFAP-, MAC-1- AND MHC II-specific
antibodies were pretreated with 0.6% hydrogen peroxide at RT
to block endogenous peroxidase then incubated overnight with
the primary antibody at 4°C. Sections reacted with the GFAP

35   antibody were incubated for 1 hr at RT with biotinylated anti-
mouse IgG made in horse (Vector Laboratories; Vectastain Elite

ABC Kit) diluted 1:200 with TBS. The sections were next
reacted for one hr with an avidin-biotin-peroxidase complex
(Vector Laboratories; Vectastain Elite ABC Kit) diluted 1:1000
with TBS. Sections incubated with the MAC-1-or MHC II-
5   specific mAβ as the primary antibody were subsequently reacted
for 1 hr at RT with biotinylated anti-rat IgG made in rabbit
diluted 1:200 with TBS, followed by incubation for one hr with
avidin-biotin-peroxidase complex diluted 1:1000 with TBS.
Sections incubated with GFAP-, MAC-1- and MHC II-specific
10   antibodies were then visualized by treatment at RT with 0.05%
DAB, 0.01% hydrogen peroxide, 0.04% nickel chloride, TBS for 4
and 11 min, respectively.

Immunolabeled sections were mounted on glass slides (VWR,
Superfrost slides), air dried overnight, dipped in Propar
15   (Anatech) and overlaid with coverslips using Permount (Fisher)
as the mounting medium.

To counterstain Aβ plaques, a subset of the GFAP-positive
sections were mounted on Superfrost slides and incubated in
aqueous 1% Thioflavin S (Sigma) for 7 min following
20   immunohistochemical processing. Sections were then dehydrated
and cleared in Propar, then overlaid with coverslips mounted
with Permount.

8.   Image Analysis

A Videometric 150 Image Analysis System (Oncor, Inc.,
25   Gaithersburg, MD) linked to a Nikon Microphot-FX microscope
through a CCD video camera and a Sony Trinitron monitor was
used for quantification of the immunoreactive slides. The
image of the section was stored in a video buffer and a color-
and saturation-based threshold was determined to select and
30   calculate the total pixel area occupied by the immunolabeled
structures. For each section, the hippocampus was manually
outlined and the total pixel area occupied by the hippocampus

was calculated. The percent amyloid burden was measured as:
(the fraction of the hippocampal area containing Aβ deposits
immunoreactive with mAβ 3D6) x 100. Similarly, the percent
neuritic burden was measured as: (the fraction of the
5   hippocampal area containing dystrophic neurites reactive with
mAβ 8E5) x100. The C-Imaging System (Compix, Inc., Cranberry
Township, PA) operating the Simple 32 Software Application
program was linked to a Nikon Microphot-FX microscope through
an Optronics camera and used to quantitate the percentage of
10   the retrospenial cortex occupied by GFAP-positive astrocytes
and MAC-1-and MHC II-positive microglia. The image of the
immunoreacted section was stored in a video buffer and a
monochrome-based threshold was determined to select and
calculate the total pixel area occupied by immunolabeled
15   cells. For each section, the retrosplenial cortex (RSC) was
manually outlined and the total pixel area occupied by the RSC
was calculated. The percent astrocytosis was defined as: (the
fraction of RSC occupied by GFAP-reactive astrocytes) X 100.
Similarly, percent microgliosis was defined as: (the fraction
20   of the RSC occupied by MAC-1- or MHC II-reactive microglia) X
100. For all image analyses, six sections at the level of the
dorsal hippocampus, each separated by consecutive 240 μm
intervals, were quantitated for each animal. In all cases,
the treatment status of the animals was unknown to the
25   observer.

Although the foregoing invention has been described in
detail for purposes of clarity of understanding, it will be
obvious that certain modifications may be practiced within the
scope of the appended claims. All publications and patent
30   documents cited herein are hereby incorporated by reference in
their entirety for all purposes to the same extent as if each
were so individually denoted.

TABLE 1

TITER AT 50% MAXIMAL O.D.

Aggregated Aβ Injected mice

Age of PDAPP	mouse 100	mouse 101	mouse 102	mouse 103	mouse 104	mouse 105	mouse 106	mouse 107	mouse 108
4	70000	150000	15000	120000	1000	15000	50000	80000	100000
6	15000	65000	30000	55000	300	15000	15000	50000	60000
8	20000	55000	50000	50000	400	15000	18000	50000	60000
10	40000	20000	60000	50000	900	15000	50000	20000	40000
12	25000	30000	60000	40000	2700	20000	70000	25000	20000

PBS Injected mice on both Immunogens at 1/100

Age of PDAPP	mouse 113	mouse 114	mouse 115	mouse 116	mouse 117
6	< 4x bkg	< 4x bkg	< 4x bkg	< 4x bkg	< 4x bkg
10	5 x bkg	< 4x bkg	< 4x bkg	< 4x bkg	< 4x bkg
12	< 4x bkg	< 4x bkg	< 4x bkg	< 4x bkg	< 4x bkg

WHAT IS CLAIMED IS:

1   1.   A pharmaceutical composition comprising an agent
2   effective to induce an immunogenic response against Aβ in a
3   patient, and a pharmaceutically acceptable adjuvant.

1   2.   The pharmaceutical composition of claim 1, wherein the
2   agent is Aβ or an active fragment thereof.

1   3.   The pharmaceutical composition of claim 1 or 2, wherein
2   the adjuvant comprises alum.

1   4.   The pharmaceutical composition of claim 1 or 2, wherein
2   the adjuvant comprises monophosphoryl lipid (MPL).

1   5.   The pharmaceutical composition of claim 1 or 2, wherein
2   the adjuvant comprises QS21.

1   6.   The pharmaceutical composition of any of the preceding
2   claims, wherein the Aβ or fragment is a component of a
3   particle.

1   7.   The pharmaceutical composition of claim 6, wherein the
2   particle is a polylactide polyglycolide copolymer (PLPG) particle.

1   8.   A method of preventing or treating a disease
2 characterized by amyloid deposit in a patient, comprising:

3   administering an agent effective to induce an immune
4 response against a peptide component of an amyloid deposit in
5 the patient.

1   9.   The method of claim 8, wherein the amyloid deposit
2 comprises aggregated Aβ peptide.

1   10.   The method of claim 8 or 9, wherein the patient is a
2 human.

1   11.   The method of any of the preceding claims, wherein the
2 disease is Alzheimer's disease.

1   12.   The method of any of the preceding claims, wherein the
2 patient is asymptomatic.

1   13.   The method of any of the preceding claims,   wherein
2 . the patient is under 50.

1   14.   The method of any of the preceding claims, wherein the
2 patient has inherited risk factors indicating susceptibility
3 to Alzheimer's disease.

1   15.   The method of any of claims 8-13, wherein the patient
2   has no known risk factors for Alzheimer's disease.

1   16.   The method of any of the preceding claims, wherein the
2   agent comprises Aβ peptide or an active fragment thereof.

1   17.   The method of any of the preceding claims, wherein the
2   agent is Aβ peptide or an active fragment thereof.

1   18.   The method of claim 17, wherein the dose of Aβ peptide
2   administered to the patient at least 50 μg.

1   19.   The method of claim 17, wherein the dose of Aβ peptide
2   administered to the patient is at least 100 μg.

1   20.   The method of any of the preceding claims, wherein the
2   Aβ peptide is Aβ42.

1   21.   The method of claim 20, wherein the Aβ peptide is
2   administered in aggregated form.

1   22.   The method of claim any of the preceding claims,
2   wherein the immune response comprises antibodies that bind to
3   the Aβ peptide.

1   23.   The method of any of the preceding claims, wherein the
2   immune response comprises T-cells that bind to the Aβ peptide
3   as a component of an MHC I or MHC II complex.

1   24.   The method of any one of claims 8, or 10-15 wherein
2   agent is an antibody to Aβ which induces an immune response by
3   binding to Aβ in the patient.

1   25.   The method of claims 8 or 10-15, wherein T-cells are
2   removed from the patient, contacted with Aβ peptide under
3   conditions in which the T-cells are primed, and the primed T-
4   cells are administered to the patient.

1   26.   The method of any of the preceding claims, wherein the
2   agent is administered orally, subcutaneously, intramuscularly,
3   topically or intravenously.

1   27.   The method of any of the preceding claims, wherein the
2   agent is administered intramuscularly or subcutaneously.

1   28.   The method of any of the preceding claims, further
2   comprising screening a library of compounds to identify a
3   compound reactive with antibodies to Aβ, and administering the
4   compound to the patient to induce the immune response.

1   29.   The method of any one of claims 8, 10-15, 26 or 27,
2   wherein the agent is an effective dose of a nucleic acid
3   encoding Aβ or an active fragment thereof, whereby the nucleic
4   acid is expressed in the patient to produce Aβ or the active
5   fragment thereof, which induces the immune response.

1   30.   The method of claim 29, wherein the nucleic acid is
2   administered through the skin.

1   31.   The method of claim 30, wherein the nucleic acid is
2   applied to the skin by a patch.

1   .32.   The method of any of the preceding claims, further
2   comprising monitoring the patient for the immune response.

1   33.   The method of any of the preceding claims, further
2   comprising administering an adjuvant that enhances the immune
3   response to the Aβ peptide.

1   34.   The method of claim 33, wherein the adjuvant and the
2   agent are administered together as a composition.

1   35.   The method of claim 33, wherein the adjuvant is
2   administered before the agent.

1   36.   The method of claim 33, wherein the adjuvant is
2   administered after the agent.

1   37.   The method of any one of claims 33-36, wherein the
2   adjuvant is alum.

1   38.   The method of any one of claims 33-36, wherein the
2   adjuvant is MPL.

1   39.   The method of any one of claims 33-36, wherein the
2   adjuvant is QS21.

1   40.   The method of anyone of claims 33-36, wherein the dose
2   of $A\beta$ peptide is greater than 10 μg.

1   41.   A method of preventing or treating Alzheimer's disease
2   comprising administering an effective dose of $A\beta$ peptide to a
3   patient.

1   42.   Use of Aβ peptide, or an antibody thereto, in the
2 manufacture of a medicament for prevention or treatment of
3 Alzheimer's disease.

1   43.   The use of claim 42, wherein the Aβ peptide is
2 combined with a pharmaceutically acceptable adjuvant in the
3 manufacture of the medicament.

1   44.   A composition comprising Aβ or a fragment linked to a
2 conjugate molecule that promotes delivery of Aβ to the
3 bloodstream of a patient and/or promotes an immune response
4 against Aβ.

1   45.   The composition of claim 44, wherein the conjugates
2 promotes an immune response against Aβ.

1   46.   The composition of claim 44 or 45, wherein the
2 conjugate is cholera toxin.

1   47.   The composition of claim 44 or 45, wherein the
2 conjugate is an immunoglobulin.

1   48.   The composition of claim 44 or 45, wherein the
2 conjugate is attenuated diphtheria toxin CRM 197.

1   49. A pharmaceutical composition comprising an agent
2   effect to induce an immunogenic response against Aβ in a
3   patient with the proviso that the composition is free of
4   Complete Freund's adjuvant.

1   50. A composition comprising a viral vector encoding Aβ or
2   a fragment thereof effective to induce an immune response
3   against Aβ.

1   51. A composition of claim 50, wherein the viral vector is
2   herpes, adenovirus, adenoassociated virus, a retrovirus,
3   sindbis, semiliki forest virus, vaccinia or avian pox.

1   52. A method of assessing efficacy of an Alzheimer's
2   treatment method in a patient, comprising

3   determining a baseline amount of antibody specific for Aβ
4   peptide in tissue sample from the patient before treatment
5   with an agent,

6   comparing an amount of antibody specific for Aβ peptide in
7   the tissue sample from the patient after treatment with the
8   agent to the baseline amount of Aβ peptide-specific antibody,

9   wherein an amount of Aβ peptide-specific antibody measured
10   after the treatment that is significantly greater than the
11   baseline amount of Aβ peptide-specific antibody indicates a
12   positive treatment outcome.

1   53.   The method of claim 52, wherein the amounts of
2   antibody are measured as antibody titers.

1   54.   The method of claim 53, wherein the amounts of
2   antibody are measured by an ELISA assay.

1   55.   A method of assessing efficacy of· an Alzheimer's
2   treatment method in a patient, comprising

3   determining a baseline amount of antibody specific for $A\beta$
4   peptide in tissue sample from a patient before treatment with
5   an agent;

6   comparing an amount of antibody specific for $A\beta$ peptide in
7   the tissue sample from the subject after treatment with the
8   agent to the baseline amount of $A\beta$ peptide-specific antibody,

9   wherein a reduction or lack of significant difference
10   between the amount of $A\beta$ peptide-specific antibody measured
11   after the treatment compared to the baseline amount of $A\beta$
12   peptide-specific antibody indicates a negative treatment
13   outcome.

1   56.   A method of assessing efficacy of an Alzheimer's
2   treatment method in a patient, comprising

3   determining a control amount of antibody specific for $A\beta$
4   peptide in tissue samples from a control population,

5   comparing an amount of antibody specific for $A\beta$ peptide in
6   a tissue sample from the patient after administering an agent
7   to the control amount of $A\beta$ peptide-specific antibody,

8   wherein an amount of Aβ peptide-specific antibody measured
9   after the treatment that is significantly greater than the
10   control amount of Aβ peptide-specific antibody indicates a
11   positive treatment outcome.

1   57. A method of assessing efficacy of an Alzheimer's
2   treatment method in a patient, comprising

3   determining a control amount of antibody specific for Aβ
4   peptide in tissues samples from a control population,

5   comparing an amount of antibody specific for Aβ peptide in
6   a tissue sample from the patient after administering an agent
7   to said control amount of Aβ peptide-specific antibody,

8   wherein a lack of significant difference between the amount
9   of Aβ peptide-specific antibody measured after beginning said
10   treatment compared to the control amount of Aβ
11   peptide-specific antibody indicates a negative treatment
12   outcome.

1   .58. A method of monitoring Alzheimer's disease or
2   susceptibility thereto in a patient, comprising:

3   detecting an immune response against Aβ peptide in a sample
4   from the patient.

1   59. The method of claim 58, wherein the patient is being
2   administered an agent effective to treat or prevent
3   Alzheimer's disease, and the level of the response determines
4   the future treatment regime of the patient.

1   60. The method of claim 59, wherein the agent is $A\beta$
2   peptide.

1   61. The method of any one of claims 57-60, wherein the
2   detecting comprises detecting an antibody that specifically
3   binds to $A\beta$ peptide.

1   62. The method of any one of claims 57-60, wherein the
2   detecting comprises detecting T-cells specifically reactive
3   with $A\beta$ peptide.

1   63. A method of assessing efficacy of an Alzheimer's
2   treatment method in a patient, comprising

3   determining a value for an amount of antibody specific for
4    $A\beta$ peptide in tissue sample from a patient who has been
5   treated with an agent;

6   comparing the value with a control value determined from a
7   population of patient experiencing amelioriation of, or
8   freedom from, symptoms of Alzheimer's disease due to treatment
9   with the agent;

10   wherein a value in the patient at least equal to the
11   control value indicates a positive response to treatment.

1   64.   Use of Aβ peptide in monitoring treatment of
2 Alzheimer's disease in a patient.

1   65.   A diagnostic kit for monitoring treatment of
2 Alzheimer's disease, comprising:

3   an agent that binds to antibodies specific for Aβ peptide.

1   66.   The diagnostic kit of claim 65, further comprising
2 labelling indicating how the kit is used for monitoring
3 treatment of Alzheimer's disease.

FIGURE 1

TITER OF AGGREGATED Aß₄₂ INJECTED PDAPP MICE OVER TIME

AGE OF PDAPP MICE (months)

Figure 2

STUDY 001
HIPPOCAMPAL AMYLOID BURDEN
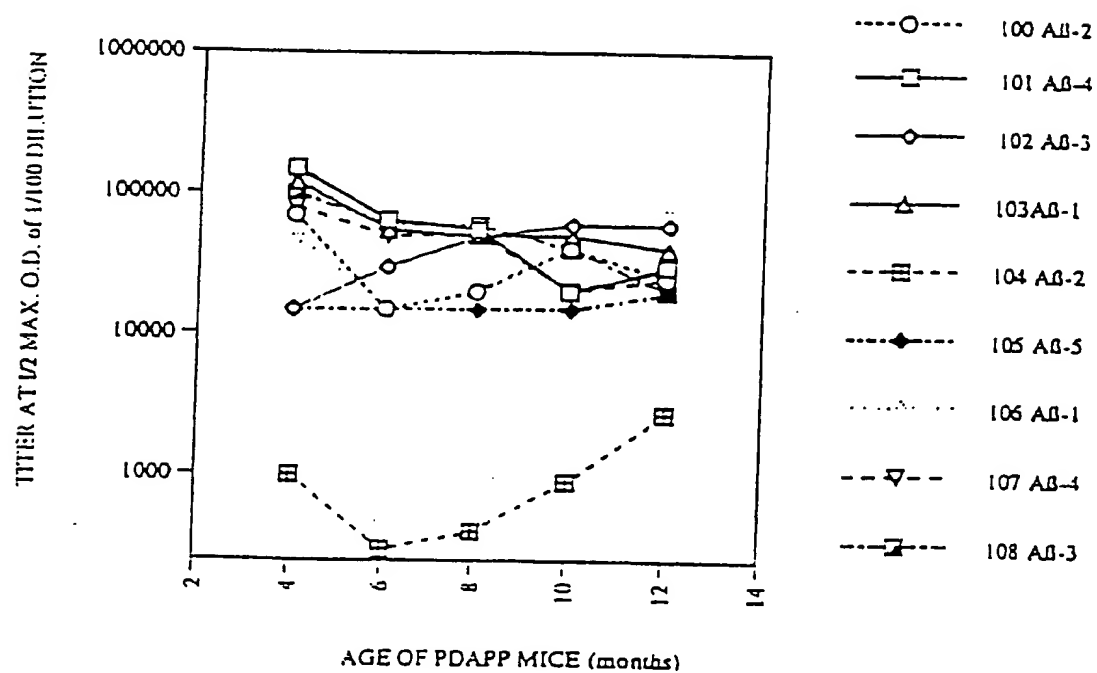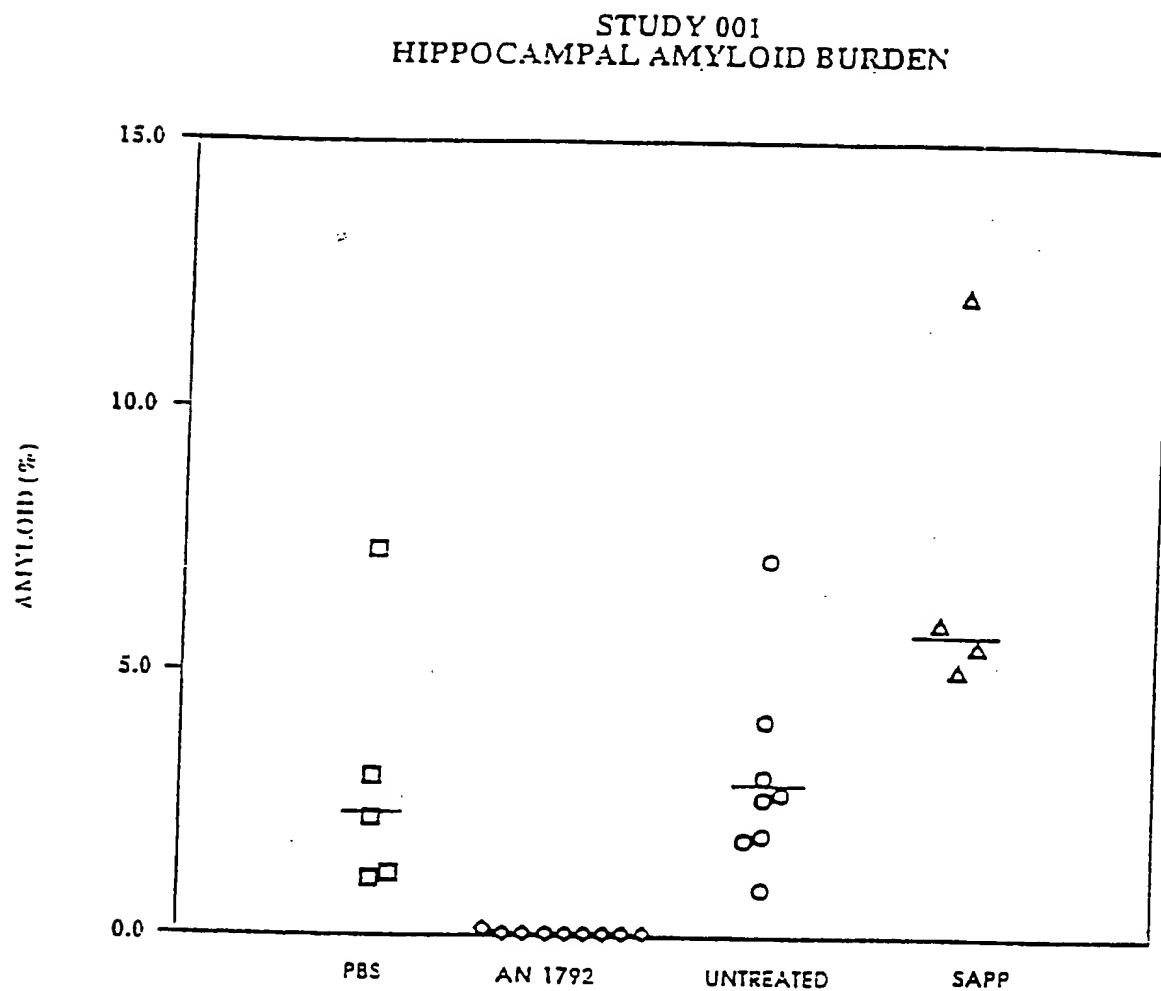
Figure 3

STUDY 001
HIPPOCAMPAL NEURITIC PLAQUE BURDEN
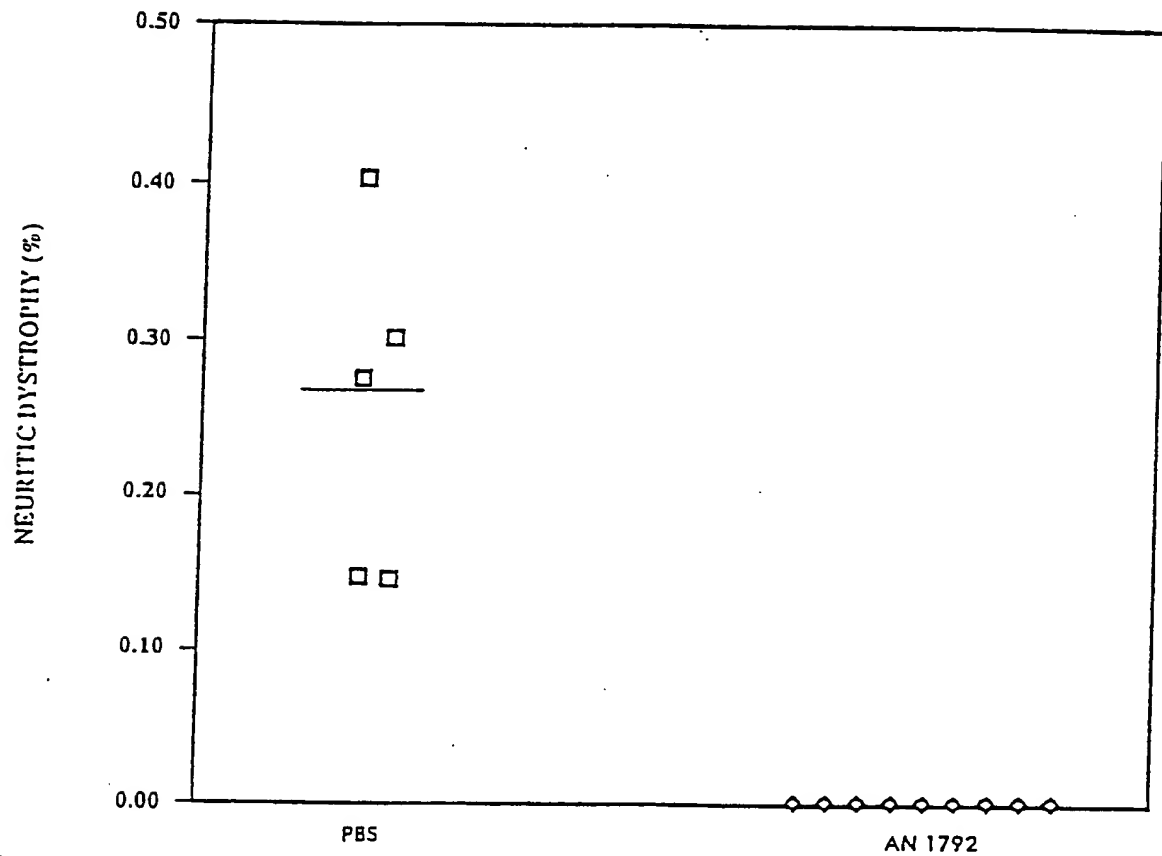
Figure 4

STUDY 001
RETROSPLENIAL CORTICAL ASTROCYTOSIS
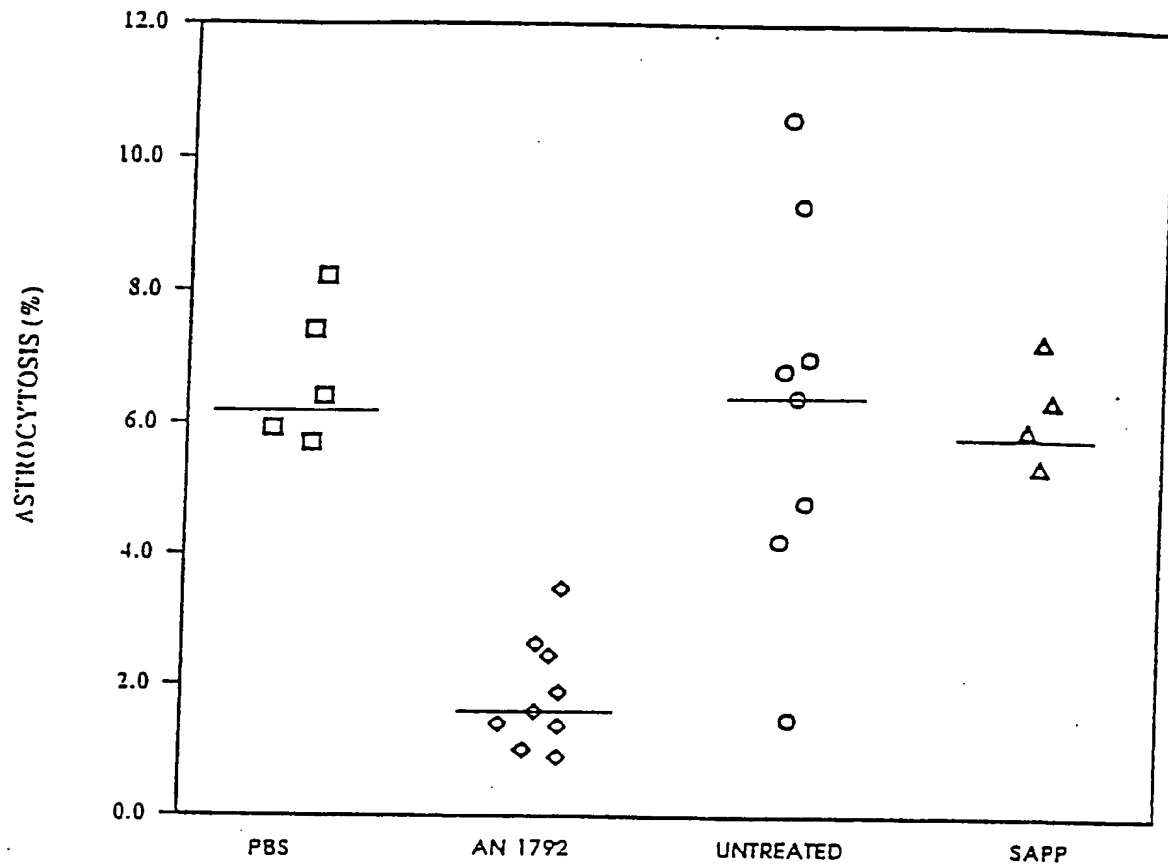
Figure 5

Antibody Titer Response to Various Doses
of AN1792 After 2, 3 and 4 Immunizations
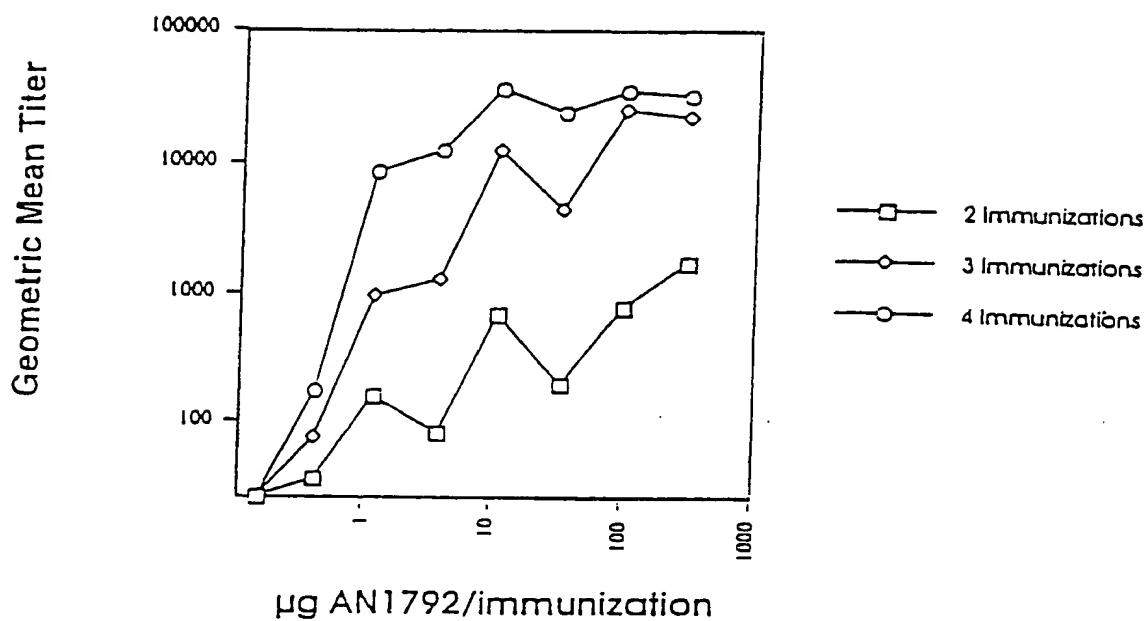
Figure 6

Kinetics of Antibody Response to AN1792

Weeks

Figure 7

STUDY 002
CORTICAL AMYLOID BURDEN
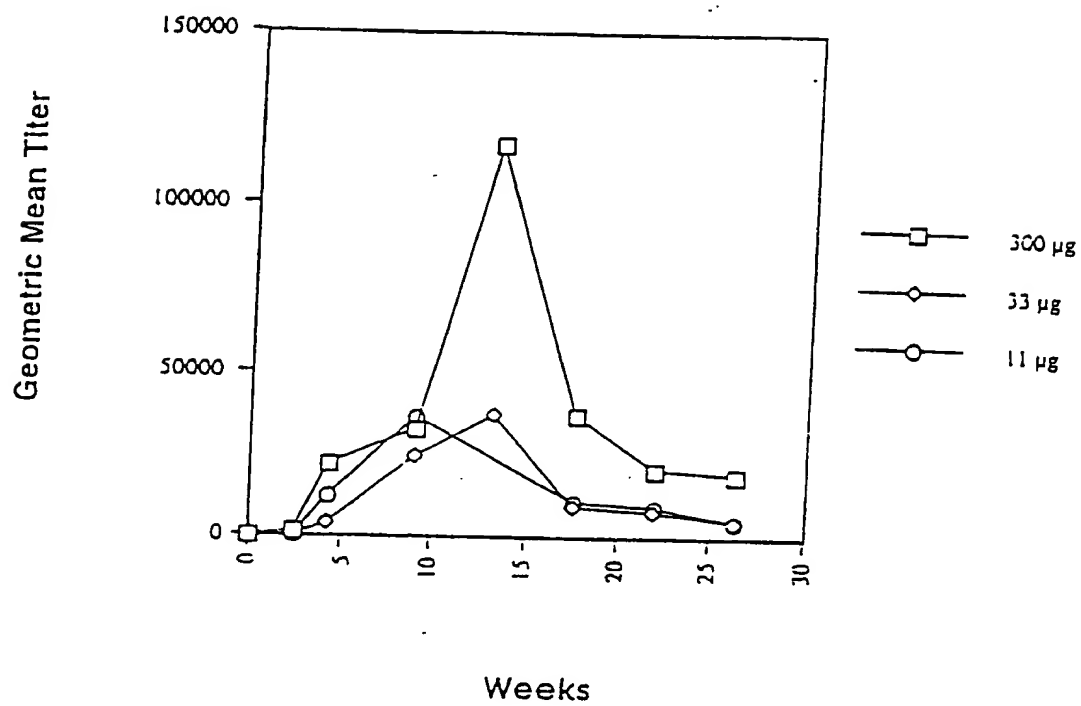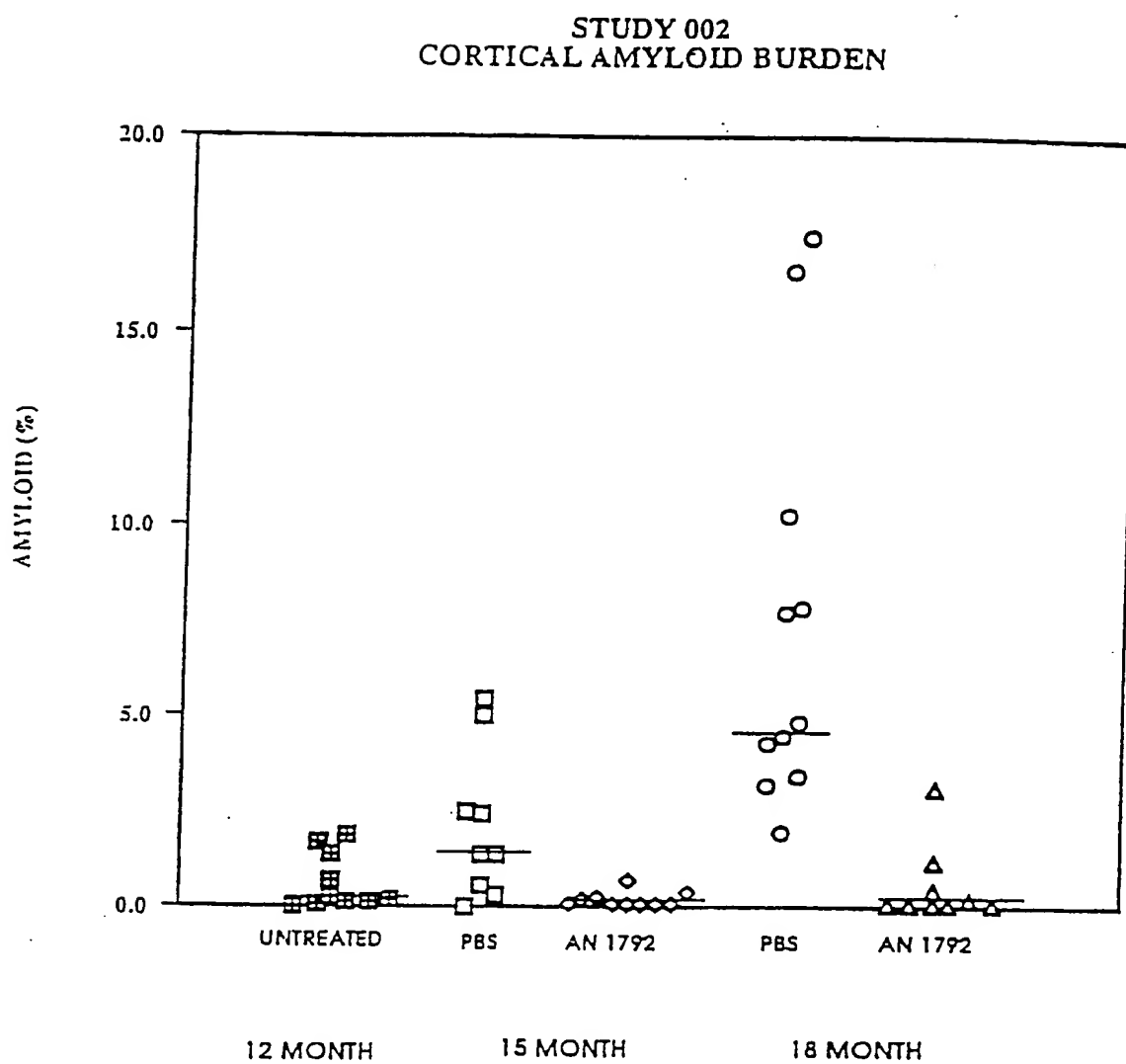
Figure 8

STUDY 002
CORTICAL NEURITIC PLAQUE BURDEN
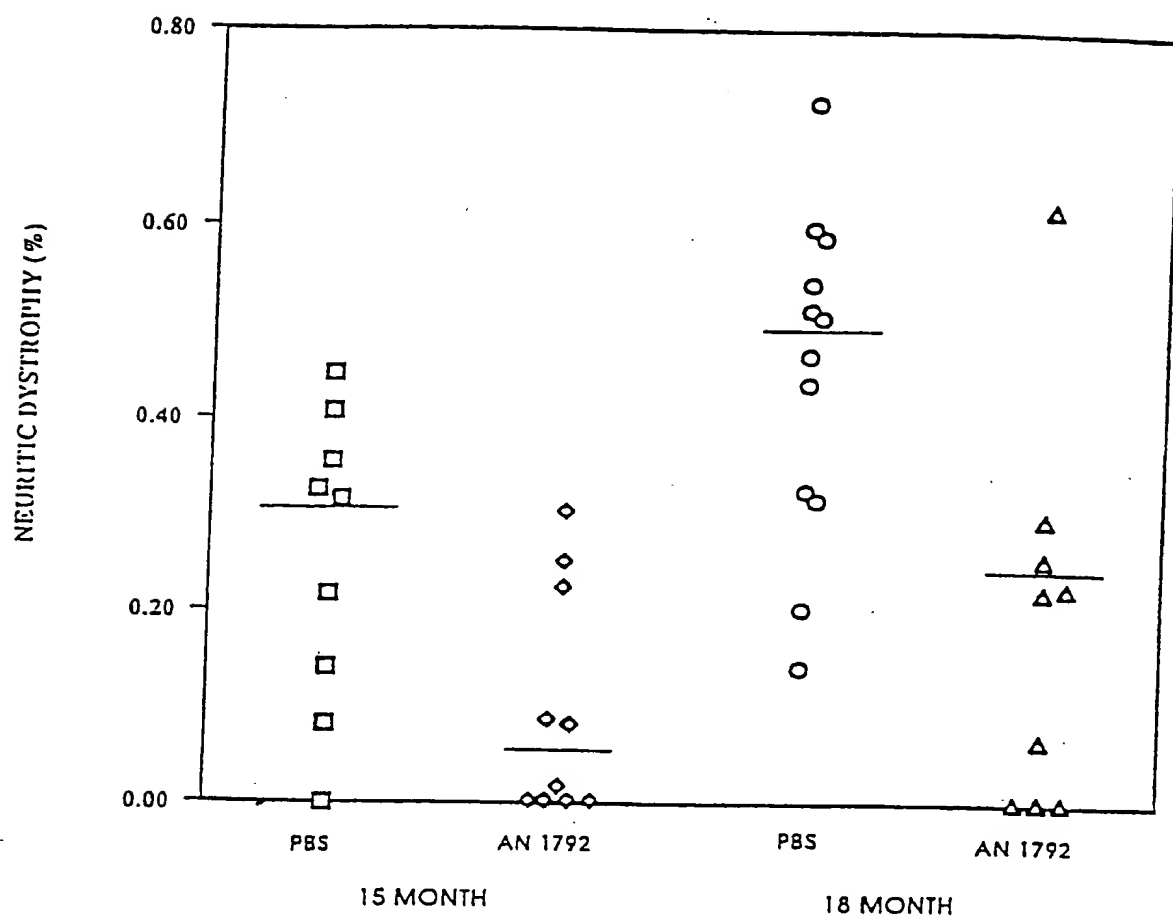
Figure 9

STUDY 002
RETROSPLENIAL CORTICAL ASTROCYTOSIS
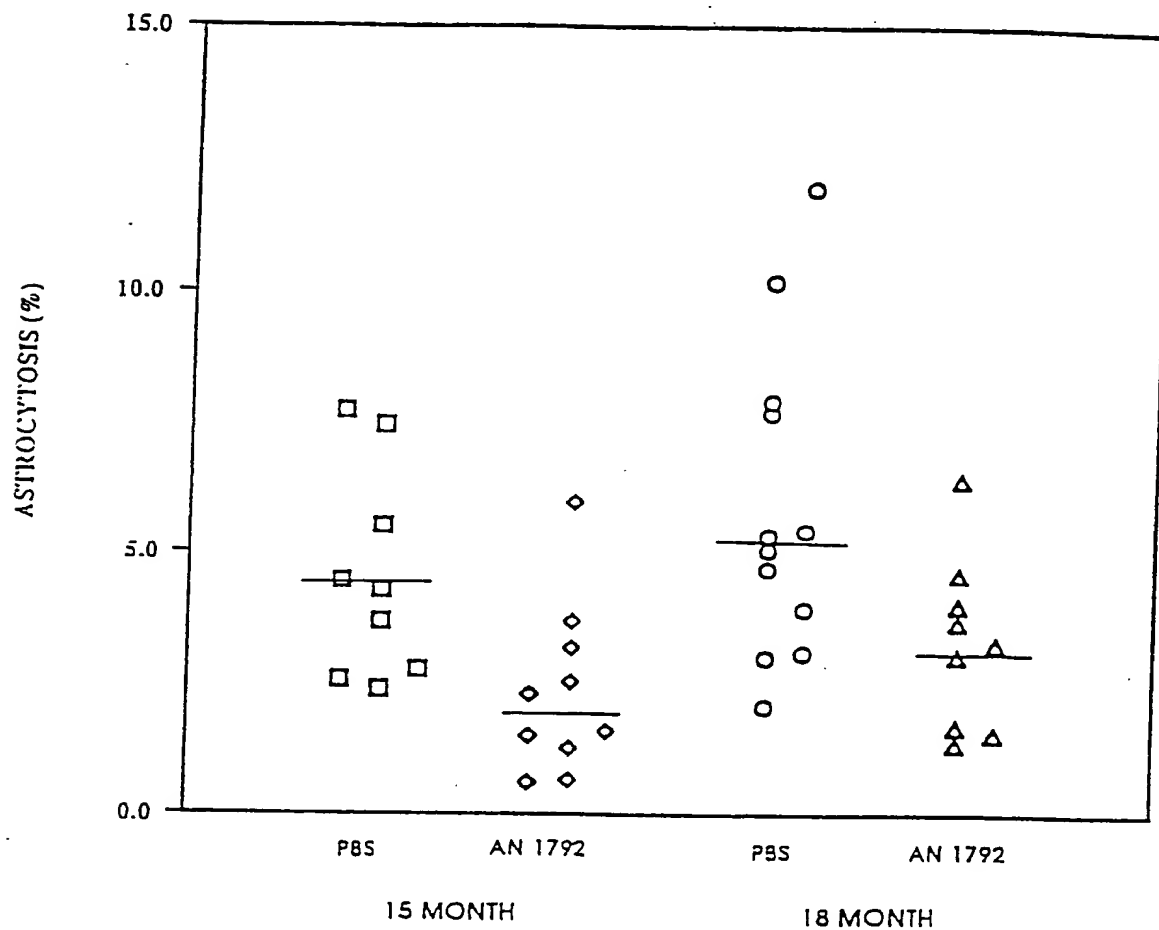
Figure 10

Study 002: Splenocyte Proliferation Assay

AN1792-Treated Group

PBS-Treated Group

Figure 11

Study 006
Therapeutic Efficacy Of Aß Fragments
Total Aß Cortical Data

Treatment Group

Figure 12

STUDY 006
CORTICAL AMYLOID BURDEN

□	PBS
◇	AGG 42
○	AGG 40
▲	1-5
⊞	1-12
◆	13-28

Figure 13

Study 006
AB1–42-Binding Antibody Titers
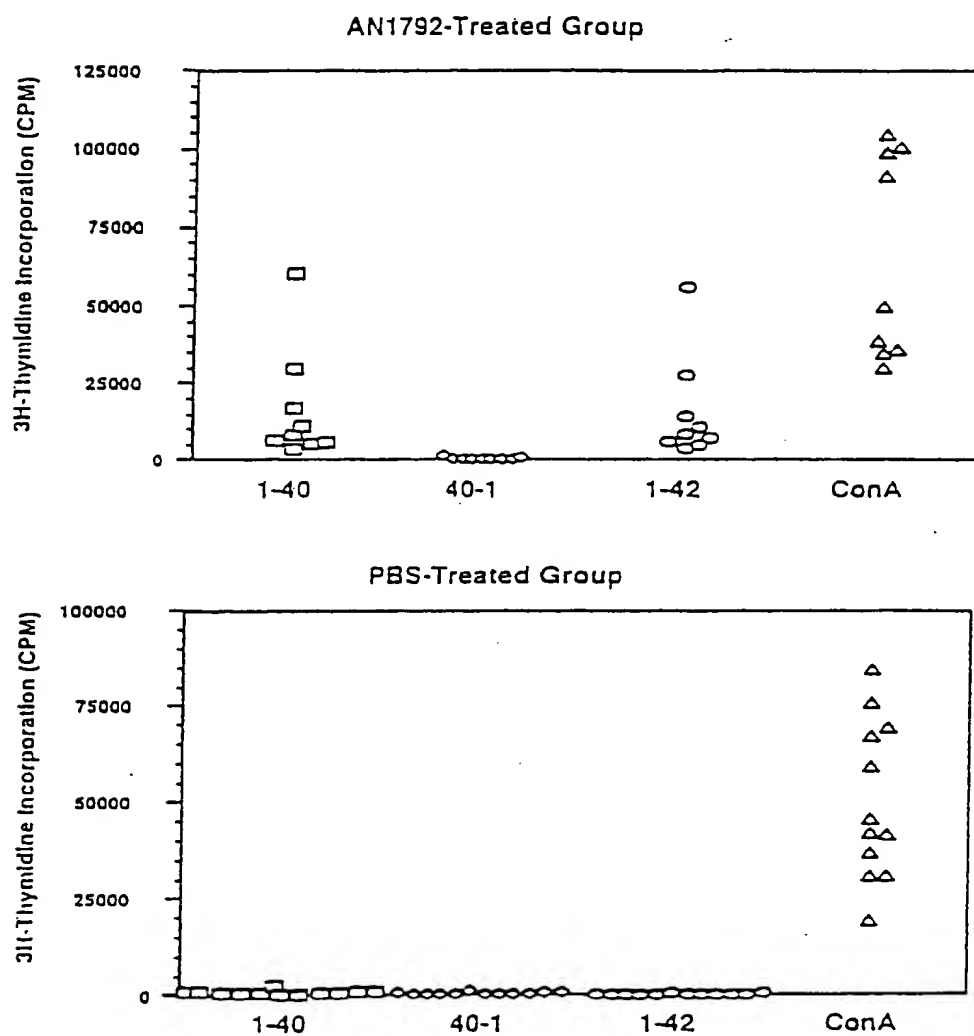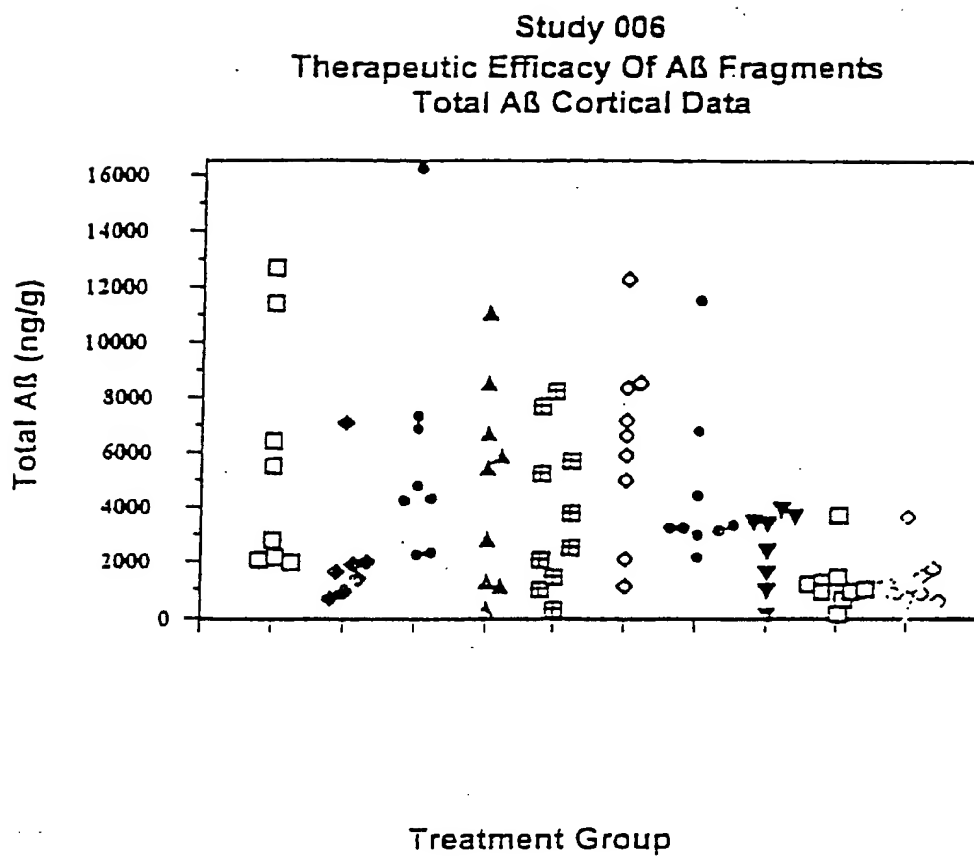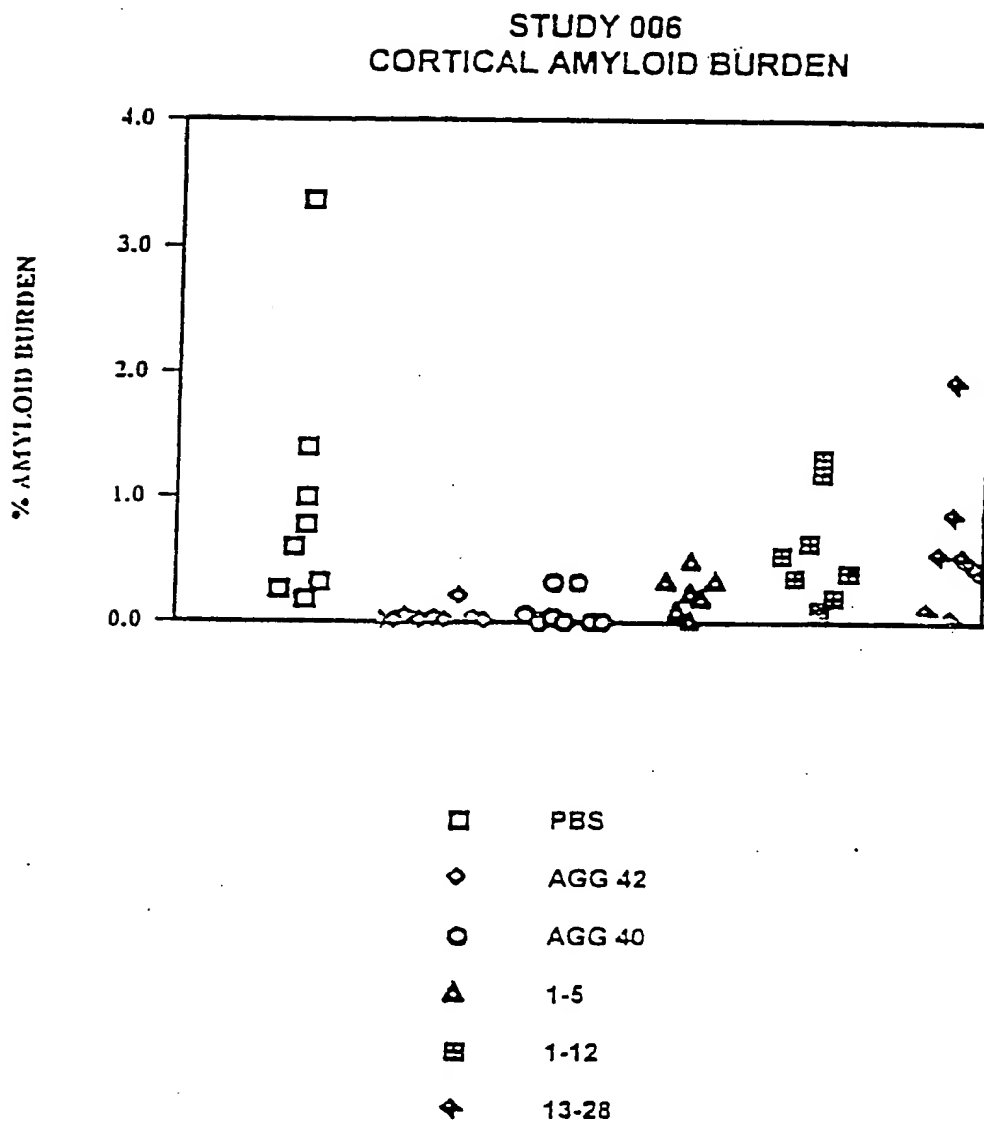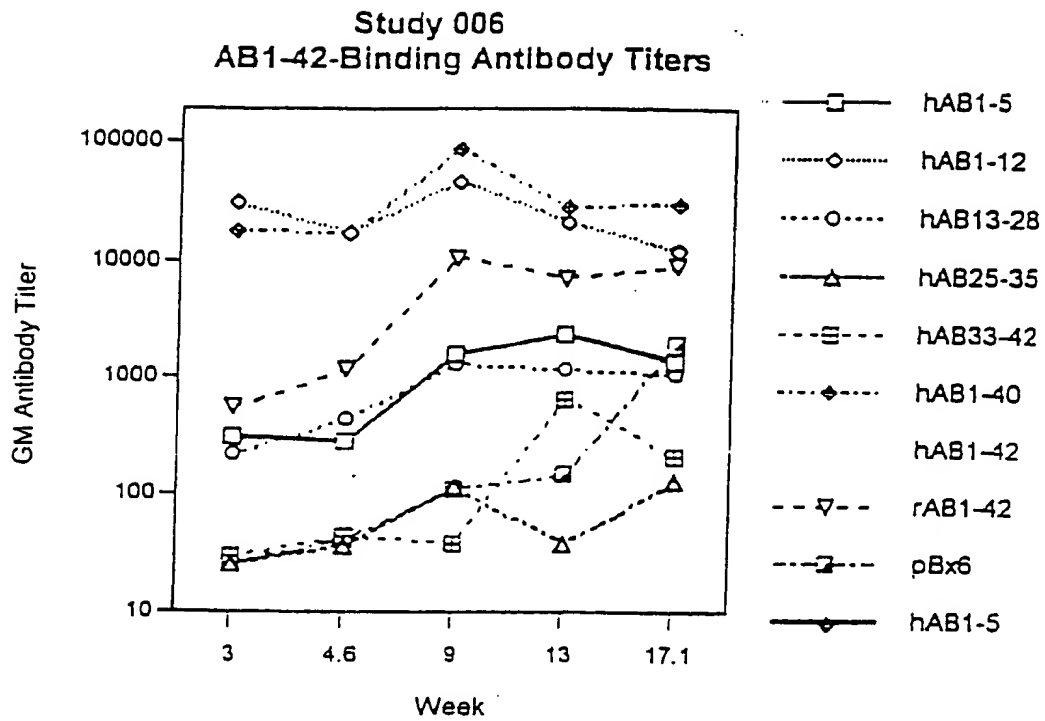
Figure 14

Antibody Responses to AN1792 With Different Adjuvants

Legend:
- 33 µg AN1792 in PBS
- 33 µg AN1792 in CFA/IFA
- 33 µg Palmitoyl-AB in Squalene
- 33 µg AN1792 in 50µg MPL/PBS
- 33µg AN1792 in Squalene
- 33 µg AN1792/50 µg MPL in Squalene
- 33 µg AN1792 in 5 mg Alum
- 300µg AN 1792 in 5 mg Alum

Y-axis: Geometric Mean Titer

X-axis: Bleed #

Figure 15

CORTEX

PBS control				2 mg Alum 100 µg AN1528		50 µg MPL 100 µg AN1528		25 µg QS21 100 µg AN1528	
Median	1817	Median	1153	Median	2031	Median	1741	Median	1199
p Value (M-W)		p Value (M-W)		p Value (M-W)		p Value (M-W)		p Value (M-W)	
Mean	1931	Mean	1825	Mean	2407	Mean	2140	Mean	1552
St. Dev.	1718	St. Dev.	1769	St. Dev.	1913	St. Dev.	1659	St. Dev.	1384
% CV	89	% CV	97	% CV	79	% CV	78	% CV	88
p Value (t Test)		p Value (t Test)		p Value (t Test)		p Value (t Test)		p Value (t Test)	
	n=16		n=15		n=20		n=21		n=21

100 µg AN1792		5 µg Thimerosal/PBS 10 µg AN1792		2 mg Alum 100 µg AN1792		50 µg MPL 100 µg AN1792		25 µg QS21 100 µg AN1792	
Median	475	Median	1687	Median	1375	Median	774	Median	950
p Value (M-W)	0.0481	p Value (M-W)		p Value (M-W)	0.5000	p Value (M-W)	0.1710	p Value (M-W)	0.4078
Mean	637	Mean	2718	Mean	1394	Mean	1192	Mean	2199
St. Dev.	655	St. Dev.	2685	St. Dev.	1166	St. Dev.	1299	St. Dev.	4187
% CV	103	% CV	99	% CV	84	% CV	109	% CV	190
p Value (t Test)	0.0106	p Value (t Test)		p Value (t Test)	0.2650	p Value (t Test)	0.1506	p Value (t Test)	0.8131
	n=15		n=15		n=21		n=21		n=16

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/25386

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6)   :A61K 38/00, 38/28, 9/26, 33/06
US CL   :424/88, 92, 570, 698; 514/ 2, 4, 21

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. :   424/88, 92, 570, 698; 514/ 2, 4, 21

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
STN: COMPOUNDS AND METHODS OF USE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EP 0 526 511 B1 (MCMICHAEL) 28 MAY 1997, see entire document	1-66
Y	Chemical Abstracts, volume 120, number 8, issued 21 February 1994, Prieels et al, "Synergistic adjuvants for vaccines" page 652, column 1, abstract no. 86406t, PCT Int. Appl. WO 94 00,153, 06 January 1994, see entire abstract	1-66

☐ Further documents are listed in the continuation of Box C.   ☐ See patent family annex.

* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	
"E" earlier document published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 MARCH 1999	0 8 APR 1999

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